THE BIOCHEMISTRY OF THE LIPIDS

BY

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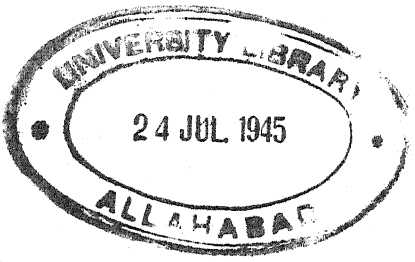
NEW YORK
JOHN WILEY & SONS, INC.
LONDON: CHAPMAN & HALL, LIMITED
1937

PRINTED IN U. S. A.

PRESS OF
BRAUNWORTH & CO., INC.
BUILDERS OF BOOKS
BRIDGEPORT, CONN.

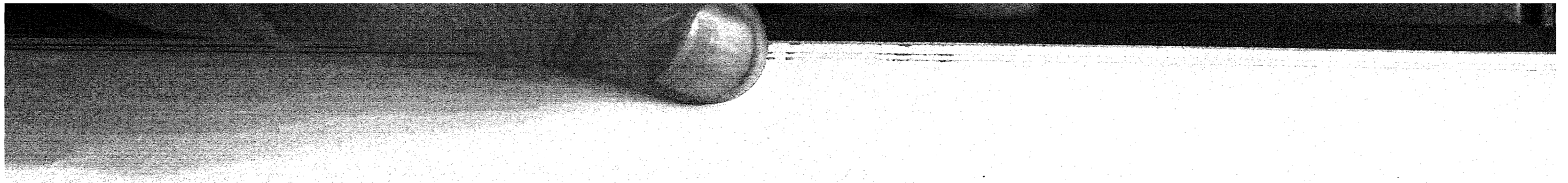


With

Affection and Respect

to

ROSS AIKEN GORTNER



PREFACE

I wish, in the beginning, to state very clearly my purpose and objective in writing this book, so that there may be no misunderstanding regarding these points. I wished to write an up-to-date, readable textbook which would attempt to gain some insight into the chemistry and physiology of the lipids. I have carefully avoided writing a technology and dictionary of fats and oils, and also I have trimmed the section on fat analysis to bare essentials. Several excellent books on these phases of the subject are available, and I have neither the ability nor the wish to attempt an improvement.

I make no claim for completeness. A great deal of available information concerning the lipids has been omitted; some of this omission, at least, has been intentional. I have had the needs of the graduate student in biochemistry constantly in mind.

This textbook was first published by the Burgess Publishing Company as a mimeographed book in its scientific series. The present work is a rearrangement, a rewriting, and an extension of that book. The original manuscript separated the chemistry and the physiology of the lipids and thus gave the appearance of a dual personality. I decided, after some hesitation, to rearrange the text and include the topics relative to physiology under the appropriate chemical headings. This has greatly increased the unity of the book.

I feel that I should present an apology for writing a book on a subject on which I have done so little research work. There are certainly a number of people much better qualified to write this book than I. Perhaps it is another case of a fool rushing in where angels fear to tread. At any rate, I wish to take this opportunity of expressing my admiration of the work of the seraphim and of singing their high praise.

In the literature citations the Journal of Biological Chemistry has been abbreviated to J.B.C. and the Journal of the American Chemical Society to J.A.C.S. Inasmuch as the above abbreviations are not those used by Chemical Abstracts it seemed best to make a note of that fact here.

I wish to thank Drs. G. O. Burr and W. R. Brown for their permission to use some of their unpublished results and for their many valuable suggestions incorporated in the section on the essential fatty acids. I

am also very grateful to Dr. A. E. Hansen for his discussion of the problem of eczema and the essential fatty acids. I am especially indebted to Dr. R. A. Gortner for his detailed criticisms; his suggestions have greatly improved the book.

I am very appreciative of the many hours that Fredrica spent on this book.

HENRY B. BULL.

October, 1936
CHICAGO, ILL.

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ERRATA

Page 5—Tiglic acid should be $C_6H_8O_2$ instead of $C_5H_8O_2$.
Linoleic acid series should be written $C_{17}H_{2n-3}COOH$ instead of $C_{18}H_{2n-3}COOH$.
Tariric acid has a triple bond in the 6–7 position.
Linolenic acid series should be written $C_{17}H_{2n-5}COOH$ instead of $C_{18}H_{2n-3}COOH$.

Page 15—Line 24—reference should be 20 instead of 02.
Line 25—dicoic acids instead of decoic acids.

Page 17—Line 15—acetoacetic for acetic.

Page 27—Lines 7 and 10—elaidin for elaiden.

Page 36—Line 19—fatty acids for fatty acid.

Page 46—Table VIII—Dermoid Cysts for Demoid Cysts.
$C_{30}H_{62}O$ for $C_{30}H_{60}O$.

Page 50—The formula for lutein has a double bonded hydroxyl group; should have single bond.

Page 52—Fig. 20—Point for 30 carbon paraffin should be omitted.

Page 53—Table IX—The second item should be *n*-heptacosane rather than *n*-hentriacontane.

Page 58—The formula for cholesterol is incorrect; carbon 5 should not have a hydrogen attached to it. Also there should be a methyl group on carbon 13 in place of the hydrogen.

Page 72—Ref. 7 should read A. Grün, Ber. **40**, 1792 (1907).

Page 84—6th line from bottom of page—Should read "one gram of the acetylated product — —"

Page 93—20th line—Glyceride molecule instead of glycerol.

Page 111—Line 30—Catechol instead of catachol.

Page 112—Table XXI—Hydroxyhydroquinone instead of hydroxydroquinone.

Page 114—Line 16—Raper for Roper.

Page 117—Line 6—71 per cent instead of 17 per cent.

Page 135—Sphingosine should have two hydroxyl groups.

Page 136—Sphingosine should have two hydroxyl groups.

THE BIOCHEMISTRY OF THE LIPIDS

INTRODUCTION AND CLASSIFICATION

Along with other branches of chemistry [1] the lipids have experienced a large growth in recent years. Fig. 1 shows the number of papers

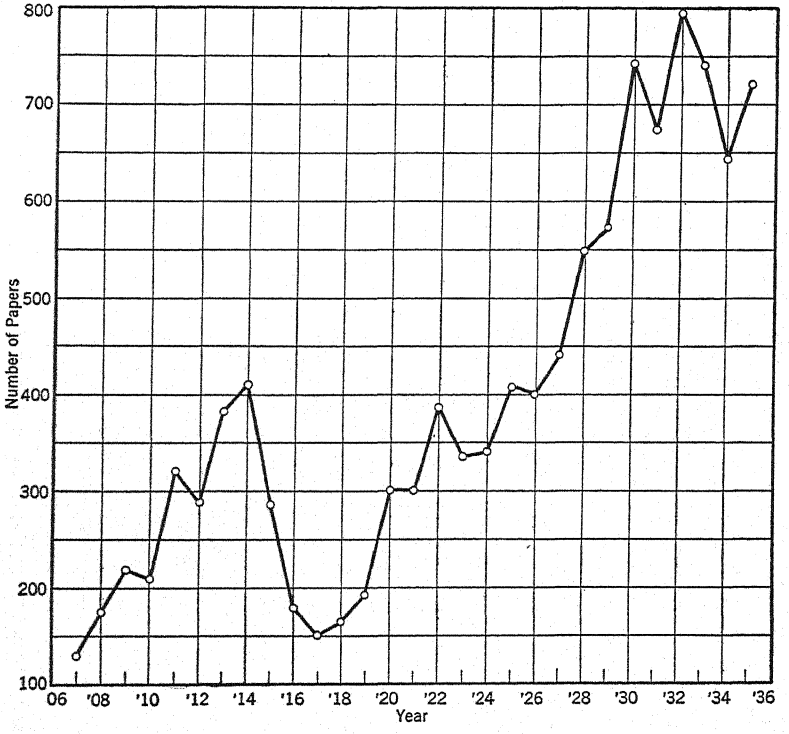


FIG. 1.—Number of papers per year published on lipids.

appearing per year in Chemical Abstracts under certain headings which were selected to include most of the papers on lipids.

We are, as far as the development of science is concerned, in the midst of an explosion. Our knowledge is increasing in an autocatalytic

[1] Edward Thomas, Science **83**, 159 (1936).

manner; in fact, we are dealing with a series of chain reactions. This explosion was interrupted by the World War; here we see the effect of an inhibitor.

Considerable ambiguity exists in the nomenclature of the lipids. Before the coinage of the word "lipid" there was really no generic name for this class of substances. Obviously the word "fat" was inadequate and the old term of lipoid had come to be used so loosely that it had lost its meaning. The author has not hesitated to take over the classification and definition proposed by Bloor [2] although his suggestions have not been officially adopted by any society.

Bloor's classification is:

LIPIDS

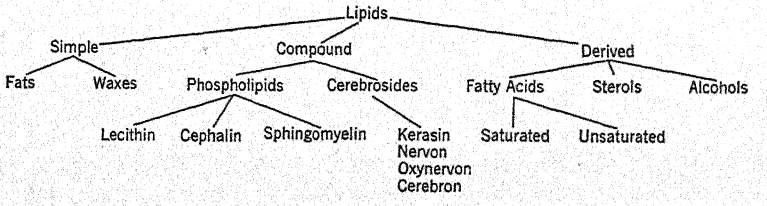
Substances having the following characteristics.

(*a*) Insolubility in water and solubility in the fat solvents, such as ether, chloroform, benzene.
(*b*) Relationship to the fatty acids as esters, either actual or potential.
(*c*) Utilization by living organisms.

I. Simple lipids—esters of fatty acids with various alcohols.
   (*a*) Fats—esters of the fatty acids with glycerol.
   (*b*) Waxes—esters of the fatty acids with alcohols other than glycerol.

II. Compound lipids—esters of fatty acid containing groups in addition to an alcohol and fatty acid.
   (*a*) Phospholipids—substituted fats containing phosphoric acid and nitrogen—lecithin, cephalin, sphingomyelin.
   (*b*) Cerebrosides—compounds of the fatty acids with a carbohydrate and containing nitrogen but no phosphoric acid—glycosides.
   (*c*) Aminolipids.
   (*d*) Sulfolipids.

III. Derived lipids—substances derived from the above groups by hydrolysis.
   (*a*) Fatty acids.
   (*b*) Sterols.
   (*c*) Alcohols.



[2] W. R. Bloor, Chem. Rev. **2**, 243 (1925).

Although the logic and simplicity of Bloor's classification make it much superior to any so-far-proposed scheme, other classifications have been adopted by certain scientific groups.

The ninth conference of the International Union of Pure and Applied Chemistry was held in July, 1928, in London. The delegates drew up a classification of the lipids and related substances and divided all the compounds into two general classes: (1) The ternary lipids, which included those substances of a lipid-like nature which contained the elements carbon, hydrogen, and oxygen, and only these elements. This class was intended to include the fats, waxes, and sterol esters. (2) The complex lipids containing other elements than the above three. The phospholipids fell into this second class. The cerebrosides were not considered to be lipids.

This conference had hardly disbanded before the National Committee of Great Britain met and proposed another classification. This committee believed that there should be not a general group but several distinct groups: (1) the esters of higher alcohols and fatty acids and the glycerides of fatty acids, and (2) the lipides which included the phospholipids and cerebrosides. The higher alcohols, sterols, and fatty acids were excluded.

Although Smedley-Maclean was a member of the British committee, apparently she was not satisfied with its findings, for she evolved a classification of her own which is used to a considerable extent by British workers. The general name is lipoids, which evidently includes all ether-soluble substances. The complex lipids are called lipins, and the phospholipids are termed phosphatides.

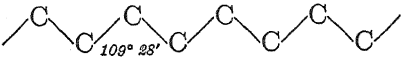
The author has been unable to find a statement of the German classification, but apparently under the general name Lipoide they include such terms as the Fette, Wachse, Phosphatide, and Cerebroside.

Some official action by the American Chemical Society should be taken to obtain a unified system of classification; especially is this desirable for proper indexing in Chemical Abstracts.

CHAPTER I

THE FATTY ACIDS

The acids of the fats and waxes are derivatives of the hydrocarbons with an open zig-zag chain. The carbon atoms form an angle of 109° 28′ between themselves.



In general, the carbon chains are unbranched and the carboxyl group is at one end.

The saturated acids are denoted by the formula $C_nH_{2n+1}COOH$; the unsaturated acids with one double bond, by $C_nH_{2n-1}COOH$; with two double bonds by $C_nH_{2n-3}COOH$, etc. Chaulmoogric acid and its homologues are exceptions, as they contain a five-membered ring. Practically all naturally occurring fatty acids have an even number of carbon atoms. The older literature reported numerous odd carbon fatty acids, but these seem to owe their existence to poor analytical technique.

TABLE I

CLASSIFICATION OF THE FATTY ACIDS

(Those which occur commonly are starred)

I. The saturated fatty acids, acetic acid series, $C_nH_{2n+1}COOH$.

Name	Carbon Number	Formula	Occurrence
Acetic	2	$C_2H_4O_2$	Vinegar
Butyric	4	$C_4H_8O_2$	Milk fat
Caproic	6	$C_6H_{12}O_2$	Butter, cocoanut and palm nut oils, etc.
Caprylic	8	$C_8H_{16}O_2$	Cocoanut and palm nut oils, butter, etc.
Capric	10	$C_{10}H_{20}O_2$	Cocoanut and palm nut oils, butter, etc.
* Lauric	12	$C_{12}H_{24}O_2$	Laurel oil, spermaceti, etc.
* Myristic	14	$C_{14}H_{28}O_2$	Nutmeg butter
* Palmitic	16	$C_{16}H_{32}O_2$	Animal and vegetable fats
* Stearic	18	$C_{18}H_{36}O_2$	Animal and vegetable fats
* Arachidic	20	$C_{20}H_{40}O_2$	Peanut oil
Behenic	22	$C_{22}H_{44}O_2$	Oil of ben, from seeds of Moringa pterygosperma
Lignoceric	24	$C_{24}H_{48}O_2$	In cerebrosides and arachis oil
Carnaubic	24	$C_{24}H_{48}O_2$	Carnauba wax
Cerotic	26	$C_{26}H_{52}O_2$	Beeswax, Chinese wax, opium wax, wool fat, etc.
Melissic	30	$C_{30}H_{60}O_2$	

TABLE I.—Classification of the Fatty Acids—*Continued*

II. The unsaturated fatty acids.

Name	Formula	Occurrence
1. Acrylic or oleic acid series.....	$C_nH_{2n-1}COOH$	
Crotonic.....	$C_4H_6O_2$	Croton oil
Tiglic.....	$C_5H_8O_2$	Croton oil
* Oleic.....	$C_{18}H_{34}O_2$	Animal and vegetable fats
Elaidic.....	$C_{18}H_{34}O_2$	Does not occur in natural fats
* Erucic.....	$C_{22}H_{42}O_2$	Rapeseed and similar oils
2. Linoleic or linolic acid series. Acids with two double bonds.	$C_{18}H_{2n-3}COOH$	
* Linoleic.....	$C_{18}H_{32}O_2$	Vegetable oils, such as linseed, cottonseed, etc.
Tariric.....	$C_{18}H_{32}O_2$	
3. Linolenic acid series. Acids with three double bonds....	$C_{18}H_{2n-5}COOH$	
* Linolenic.....	$C_{18}H_{30}O_2$	Linseed oil
Elaeostearic.....	$C_{18}H_{30}O_2$	Chinese wood oil
4. Series. Acids with four double bonds.....	$C_nH_{2n-7}COOH$	
* Clupanodonic.....	$C_{18}H_{28}O_2$	Japanese sardine oil
* Arachidonic.....	$C_{20}H_{32}O_2$	Lecithin, cephalin

III. Saturated monohydroxy acids.

Name	Formula	Occurrence
α-Hydroxy propionic.....	$C_3H_6O_3$	Metabolic intermediate
β-Hydroxy butyric	$C_4H_8O_3$	Metabolic intermediate
Hydroxy-*n*-decanic.....	$C_{10}H_{20}O_3$	Brain phospholipides
Lanopalmic.....	$C_{16}H_{32}O_3$	Wool fat
Cerebronic.....	$C_{24}H_{48}O_3$	Cerebron

IV. Unsaturated monohydroxy acids, $C_nH_{2n-2}O_3$.

Name	Formula	Occurrence
Ricinoleic.....	$C_{18}H_{34}O_3$	Castor oil

TABLE I.—CLASSIFICATION OF THE FATTY ACIDS—*Continued*

V. Saturated dihydroxy acids, $C_nH_{2n}O_4$.

Name	Formula	Occurrence
Dihydroxystearic	$C_{18}H_{36}O_4$	Castor oil
Lanoceric	$C_{30}H_{60}O_4$	Wool fat

VI. Keto acids.

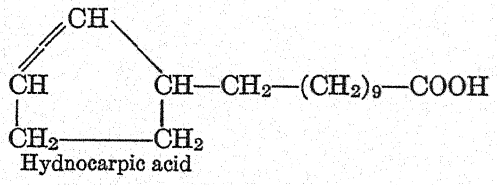
Name	Formula	Occurrence
Pyruvic	$C_3H_4O_3$	Metabolic intermediate
Acetoacetic	$C_4H_6O_3$	Metabolic intermediate

VII. Saturated dibasic acids, $C_nH_{2n-2}O_4$.

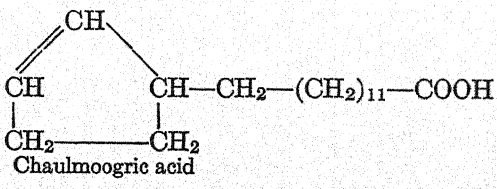
Name	Formula	Occurrence
Japanic	$C_{22}H_{42}O_4$	Japan wax

VIII. Chaulmoogric series. Cyclic acids with one double bond (used in treatment of leprosy).

Name	Formula	Occurrence
Hydnocarpic	$C_{16}H_{28}O_2$	Chaulmoogra oil
Chaulmoogric	$C_{18}H_{32}O_2$	Chaulmoogra oil



Hydnocarpic acid



Chaulmoogric acid

The acids with four or less carbon atoms are miscible in water in all proportions; those having more than ten carbon atoms are insoluble.

Except for a few of the short-chain members, they are not generally found in the free state in living cells and their presence would indicate partial hydrolysis. The lower members are liquid with an unpleasant odor and taste. The higher members are solid at room temperature and are odorless and tasteless.

Most of the reactions of the fatty acids are well known. The hydrogen of the carboxyl group can be replaced by a metal to form a soap. Esters are easily formed by splitting out water between the acid and alcohol with the formation of waxes if the alcohol is monoatomic and has a high molecular weight or a fat or oil if the alcohol is glycerol.

The carboxyl group may be reduced in the presence of nickel catalyst and hydrogen at 200 atmospheres and 320° C. to form the corresponding alcohol. This is an important reaction commercially. The alcohol is then either (1) heated with H_2SO_4 to 100° C. in the presence of an acid anhydride such as acetic anhydride or (2) it may be treated with chlorosulfuric acid at 30° C., or (3) fuming H_2SO_4 may be used at a higher temperature. This treatment gives rise to the sulfated alcohol which has the structure RCH_2OSO_3H. These compounds are excellent detergents and are coming into general use.[1] They possess the advantage over soaps that their magnesium and calcium salts are soluble and accordingly are not affected by hard water. Another advantage is that they are stable in acid solutions. The sodium salt is most often used. The source of the fatty acid as starting material is usually hydrogenated cocoanut oil or palm kernel oil.

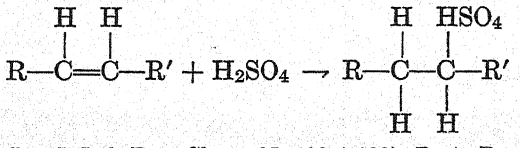
In addition to the sulfonated alcohols, another type of detergent is made by causing isethionic acid or its salt to combine with oleic acid or its derivative, resulting in

$$[CH_3(CH_2)_7 \cdot CH : CH(CH_2)_7 \cdot COOCH_2CH_2SO_3Na].$$

This product cannot be used with alkali, as a soap is formed.

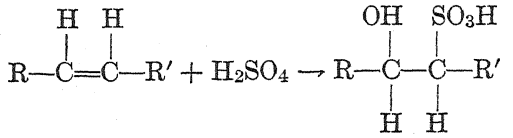
It has long been the commercial practice to treat oils with sulfuric acid, by which means a soluble product is formed. They are used as detergents. Concentrated sulfuric acid is used and hydrolysis is thus avoided. There are two sets of reactions, depending upon the temperature employed.

Below 100° C., strong acids are formed and these are undesirable:



[1] D. H. Killeffer, J. Ind. Eng. Chem. **25**, 138 (1933); R. A. Duncan, *ibid.* **26**, 24 (1934).

Above 100° C., however, true sulfonic acids are obtained:



The sulfuric acid attacks only double bonds or hydroxy groups. After treatment with the acid the oil is boiled with water and hydrolyzed.

Upon electrolyzing a fatty acid or soap, a series of reactions occur, certain of which are favored by particular conditions:

$$2CH_3CH_2COOH \rightarrow 2CH_3CH_2COO^- + H_2$$
$$2CH_3CH_2COO^- + H_2O \rightarrow 2CH_3CH_2COOH + O_2$$
$$2CH_3CH_2COO^- \rightarrow CH_3CH_2CH_2CH_3 + CO_2$$
$$2CH_3CH_2COO^- \rightarrow CH_3CH_2COOCH_2CH_3 + CO_2$$
$$2CH_3CH_2COO^- \rightarrow CH_2 : CH_2 + CH_3CH_2COOH$$

Fatty acids higher than propionic form ketones spontaneously on exposure to light. They are formed with ease if the fatty acid is heated with iron filings.[2] For example, when stearic acid is heated at 280° C. for two hours in the presence of iron, about 70 per cent yield of stearone, $(C_{17}H_{35})_2CO$, is formed.

Substitution Products. Frequently, it is desired to substitute certain groups or elements into the paraffin chain. It is comparatively easy to make an alpha substitution. Ward[3] was able to use red phosphorus to catalyze the bromination of aliphatic acids in the alpha position. With this technique, adequate amounts of halogenated acid may be obtained. This acid may be used as the starting material for the preparation of the corresponding α-hydroxy acid by treating the brominated acid with KOH or for the amino acid with the use of NH_3 or in the synthesis of the polypeptides. It is not an easy matter to substitute in positions other than the α. Special reactions must be used for the product desired.

An exact measure of the extent of a chemical reaction is to be found in the free energy change involved. The following free energy values have been taken from the monograph by Parks and Huffman.[4]

[2] S. H. Piper, A. C. Chibnall, S. J. Hopkins, A. Pollard, J. A. B. Smith, and E. F. Williams, Biochem. J. **25**, 2072 (1931).

[3] C. F. Ward, J. Chem. Soc. **121**, 1161 (1922).

[4] G. S. Parks and H. M. Huffman, The Free Energies of Some Organic Compounds, Chemical Catalog Co., New York, 1932.

TABLE II

FREE ENERGIES OF SOME ORGANIC COMPOUNDS BY PARKS AND HUFFMAN

(A. C. S. Monograph)

	Calories
Insertion of CH_2 into a hydrocarbon chain	+ 1,080
Substitution of CH_3 for H attached to a main hydrocarbon chain	+ 1,900
Conversion of a simple bond into an ethylenic double bond	about +20,000
Substitution of OH for H to form a monohydroxy primary alcohol	−34,000
Substitution of O for 2H to form a ketone	−30,000
Substitution of COOH for H	−83,200
Substitution of NH_2 for H to form an amine	+ 6,000
Substitution of NO_2 for H to form a nitro compound	+ 7,000
Substitution of Cl for H	− 1,600
Substitution of Br for H	+ 4,500
Substitution of I for H	+10,000

These data are to be interpreted in the light of the equation

$$-\Delta F = RT \ln K$$

where ΔF is the free energy involved in the reaction, K is the equilibrium constant, R is the gas constant, and T is the absolute temperature. A positive free energy means that the reaction proceeds with difficulty; i.e., equilibrium occurs at a small concentration of the substance. A negative free energy, on the other hand, means that equilibrium is obtained only after considerable amounts of the material have been formed. The number of calories is the measure of the extent of these reactions either positively or negatively. The free energy change has nothing to do with the speed of the reactions.

Synthesis. There are several ways of synthesizing the various fatty acids. Bleyberg and Ulrich [5] used the malonic ester in the following set of reactions to prepare a fatty acid with two more carbon atoms than the original acid.

$$RCOOH + C_2H_5OH \rightarrow RCOOC_2H_5 \xrightarrow{Na} RCH_2OH$$

$$RCH_2OH \xrightarrow{HI} RCH_2I \xrightarrow{\text{Na malonic ester}} RCH_2CH(CO_2Et)_2$$

$$RCH_2CH(CO_2Et)_2 \xrightarrow{\text{Hydrolysis}} RCH_2CH(COOH)_2$$

$$RCH_2CH(COOH)_2 \xrightarrow{\text{Heat}} RCH_2CH_2COOH$$

[5] W. Bleyberg and H. Ulrich, Ber. 64B, 2504 (1931).

Francis, Piper, and Malkin[6] prepared the higher fatty acids by reducing the original fatty acid with hydrogen under pressure and at a higher temperature and then halogenating the alcohol which was reacted with hydrogen cyanide followed by saponification.

Levene and Taylor[7] employed a method similar to the above but the ethyl ester was reduced with metallic sodium while dissolved in ethyl alcohol.

Fordyce and Johnson[8] made use of the selective action of alkyl magnesium halides upon sebacyl chloride and 9-carbethoxynonoyl chloride to prepare the straight- and branched-chain ketonic acids of high molecular weight (C_{14}—C_{18}). They were thus able to add ten carbon atoms to a carbon chain all at one time. The iso- as well as the normal acids were prepared by the reduction of the corresponding ketonic acids.

Fatty Acid Synthesis in Vivo. It is possible to follow the synthesis of the fatty acids *in vitro* step by step. Unfortunately, this is not true for the synthesis *in vivo,* and we have scarcely progressed beyond the armchair stage.

It was shown by Lawes and Gilbert[9] as long ago as 1866 that in hogs carbohydrates definitely give rise to fats. Although the feeding of carbohydrates increases the fat stores of the body, the amount of fat in the liver is not increased to any considerable extent. This seems to indicate that the liver is not involved in the conversion of carbohydrate to fat. Exactly where this conversion takes place is not known, though the natural assumption is that the reaction proceeds in the cells in which the fat is deposited.

There are, in general, two approaches to the problem of the conversion of carbohydrates to fat. One can assume with Emil Fischer that three molecules of glucose condense to form stearic acid, or two pentoses and one hexose molecule condense to form palmitic acid. Such a reaction seems unlikely, and it is not clear why three molecules of pentoses do not combine to form a fatty acid containing fifteen carbon atoms. The other approach is to consider certain fragments which are known to be derivable from carbohydrate sources and to speculate on the possible combination of these to form the higher fatty acids.

Numerous theories have been put forward by various authors. The three favorite starting compounds have been acetaldehyde, pyruvic acid, and aldol. Actually, these three compounds are more or less

[6] F. Francis, S. H. Piper, and T. Malkin, Proc. Roy. Soc. **128**, 214 (1930).
[7] P. A. Levene and F. A. Taylor, J.B.C. **59**, 907 (1924).
[8] C. R. Fordyce and J. R. Johnson, J.A.C.S. **55**, 3368 (1933).
[9] Lawes and Gilbert, Phil. Mag. Dec., 1866.

equivalent since they can be derived from each other fairly easily. For example, pyruvic acid is converted into acetaldehyde by a decarboxylase from yeast, $CH_3COCOOH \rightarrow CH_3CHO + CO_2$; and acetaldehyde condenses to aldol at ordinary temperatures in the presence of a trace of alkali, $2CH_3CHO \rightarrow CH_3CH(OH)CH_2CHO$. It is possible that aldol rearranges to yield butyric acid or else condenses with another molecule of aldol or acetaldehyde and then rearranges to form a fatty acid. These reactions have been carried out *in vitro*, but their extent *in vivo* is not known. Smedley-Maclean[10] seems to favor pyruvic acid as an intermediate. Rather unexpectedly, yeast gives the highest yields of fat in the presence of sodium acetate and ethyl alcohol and only then when the media are oxygenated. With the other suggested intermediates very little, if any, fat is obtained. Of course, sugar leads to an accumulation of fat.

The problem of the intermediates in the conversion of carbohydrates is still completely unsolved, and the solution does not seem in sight. Any theory must account for the occurrence of only even-numbered carbon atoms in the molecule and also the general absence of short-chain fatty acids.

Incidentally the reaction of glucose to stearic acid

$$5C_6H_{12}O_6 + 4O_2 \rightarrow C_{18}H_{26}O_2 + 12CO_2 + 12H_2O$$

involves a free energy change of $-945{,}720$ calories.

It is found in feeding experiments that carbohydrates give rise principally to palmitic, stearic, and oleic acids.

Proteins to Fatty Acids. The question naturally arises as to the possibility of the conversion of protein to fatty acids. It is known that some of the amino acids are convertible to carbohydrates, and thus it would seem reasonable to assume that protein can act as precursor of fatty acid. The conversion of protein to carbohydrates is shown in phlorhizin diabetes, when the body is not able to retain its glucose. The glucose and glycogen stores are exhausted, and then the protein as well is called in. Such of the amino acids as are capable of being converted into glucose are so converted and promptly excreted. When the loss of glucose is complete, the D : N (dextrose : nitrogen) ratio is 3.65.

The Degradation of Fatty Acids. It is possible to degrade a fatty acid one carbon at a time by distilling its calcium salt with calcium acetate and oxidizing the ketone which is formed with dilute sulfuric acid and dichromate, which results in a fatty acid with one less carbon atom than the original acid.

[10] Ida Smedley-Maclean, Ergebnisse Enzymforschung V, 285 (1936).

Conversion of Fatty Acid to Carbohydrate. This reaction has been demonstrated to occur in the germinating castor bean.[11] The evidence for the conversion of fat to carbohydrate is of two kinds: (1) actual estimation of the quantity of fat and carbohydrate over a period of time which, in a closed system such as a germinating castor bean, is absolute and conclusive evidence for the conversion of fat to carbohydrate; (2) study of the respiratory quotient $\left(\text{R. Q.} = \frac{CO_2}{O_2}\right)$. Just as an R. Q. above 1 indicates the conversion of carbohydrates to fats, so one below 0.71 would indicate the conversion of fats to carbohydrates. Such a value is dangerous to interpret. It may mean that the body is retaining the CO_2 for a time, and such experiments should be conducted over a long period. R. Q.'s below 0.71 have been reported for hibernating animals, which possibly means that the animals were burning fat and converting the glycerol into sugar. Likewise, in severe diabetes quotients below 0.70 have been observed.

Gemmill and Holmes[12] were able to demonstrate the formation of carbohydrate from fat in slices of rat's liver.

Oxidation of Fatty Acids. Fatty acids are quickly and quantitatively oxidized to CO_2 and H_2O by a hot sulfuric acid-dichromate mixture. A milder oxidation with dichromate results in α-oxidation. Dakin was able to demonstrate β-oxidation when hydrogen peroxide was used as the oxidizing agent.

It is well known that the α-carbon atom of an aliphatic chain is most reactive; accordingly, one might expect that α-oxidation would be predominately the mode of oxidation, and furthermore, according to Popoff's rule, the oxidation of a hydroxy acid continues at the point at which it has been initiated, *i.e.*, on the α-carbon atom. It is, therefore, surprising that we should encounter what appears to be β-oxidation in the animal body. Witzemann[13] has studied this problem by oxidizing various hydroxy acids with potassium permanganate in a neutral or faintly alkaline medium. He found two reactions to take place which were as follows:

one carbon atom is lost:

$$(A) \qquad RCH_2CHOHCOOH + 2O \rightarrow RCH_2COOH + H_2O + CO_2$$

and two carbon atoms are lost:

$$(B) \qquad RCH_2CHOHCOOH + 5O \rightarrow RCOOH + 2H_2O + 2CO_2$$

[11] J. R. Murlin *et al.*, J. Gen. Physiol. **17**, 283 (1933).
[12] C. L. Gemmill and E. G. Holmes, Biochem. J. **29**, 338 (1935).
[13] E. J. Witzemann, J.B.C. **95**, 219 (1932).

In reaction (*A*) the acid has undergone α-oxidation, whereas reaction (*B*) shows β-oxidation. Fig. 2, a plot of Witzemann's data, indicates the extent of each reaction as a function of the length of the fatty acid chain.

The results show definitely that β-oxidation predominates for the longer carbon chain and that Popoff's rule is no longer valid. The presence of alkali was also found to increase the amount of β-oxidation. Witzemann explains this shift from α- to β-oxidation by suggesting that fatty acids enolize in the following manner:

$$\begin{array}{llll} CH_3 & CH_3 & CH_3 & CH_3 \\ | & | & | & | \\ CH_2 & CH_2 & CH & COONa \\ | & | & \| & \\ CHOH & \rightarrow CO & \rightarrow COH & \rightarrow \\ | & | & | & \\ COONa & COONa & COONa & 2CO_2 + 2H_2O \end{array}$$

The initial step is therefore α-oxidation and is in keeping with our knowledge of organic chemistry.

Clutterbuck and Raper[14] investigated the oxidation of the ammonium salts of the normal saturated fatty acid with hydrogen peroxide at 90° C. Even at this elevated temperature the reaction proceeds slowly, and at the end of four hours only about 30 per cent of the acid had undergone oxidation. β-, γ-, and δ-oxidations of stearic, palmitic, and myristic acid were observed, and to about the same extent in each of the acids. Ketonic but no hydroxy acids were isolated from the oxidized products, and the authors conclude that the first step in the oxidation is the formation of a ketonic and not a hydroxy acid. After oxidizing caproic, heptoic, and caprylic acids the corresponding γ- and δ-ketonic acids were isolated as semicarbazones.

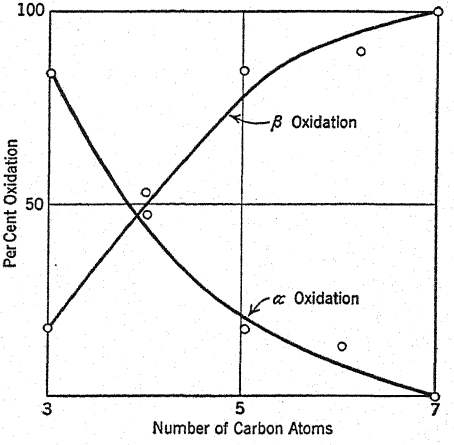


FIG. 2.—Oxidation of hydroxy fatty acids with potassium permanganate.

[14] P. W. Clutterbuck and H. S. Raper, Biochem. J. **19**, 385 (1925).

Clutterbuck and Raper point out the possible importance of δ-oxidation because by this mode of oxidation succinic acid might result which would explain how fatty acids might give rise to carbohydrates. Succinic acid is easily converted into fumaric and malic acids in muscle and liver tissue, and malic acid, in phlorhizin diabetes, is converted into glucose. This scheme would provide a method by which fatty acids could be utilized in muscle work.

Stewart, Gaddie, and Dunlop[15] were able to demonstrate that fat is apparently used in muscular work because after a certain amount of work had been done by normal men, during which time the respiratory quotient was unity, the respiratory quotient began to fall, and fell progressively to 0.85 as the work was continued. No protein was used, and the necessary conclusion is that fat must be utilized in muscular work.

Smedley-Maclean and Pearce[16] oxidized fatty acids with hydrogen peroxide in the presence of a small amount of $CuSO_4$ as a catalyst. They found the reaction to proceed with ease. Contrary to Clutterbuck and Raper, they were unable to detect ketonic acids.

Oxidation in Vivo. More is known about the oxidation of fatty acids *in vivo* than about their synthesis, but even so, our knowledge is still meager. Whatever may be the manner in which the body oxidizes fatty acid, the advantage of fat as a fuel is obvious. One gram of fat yields 9.3 calories; a monosaccharide gives 3.75 calories; a disaccharide, 4.23 calories; protein, 5.7 calories in a bomb but only 4.1 calories in the body.

Normally, the fatty acids are oxidized completely to carbon dioxide and water and no traces of the intermediates are left. Knoop[17] conceived the idea of feeding to animals the phenyl derivatives of the lower fatty acids. It is well known that benzoic acid is not oxidized in the body, and when fed, is excreted in the urine partly in combination with glycine as hippuric acid.

$$C_6H_5COOH + CH_2NH_2COOH \rightarrow C_6H_5COHNCH_2COOH + H_2O$$

Phenylacetic acid, however, forms phenaceturic acid:

$$C_6H_5CH_2COOH + CH_2NH_2COOH \rightarrow C_6H_5CH_2CONHCH_2COOH + H_2O$$

Phenylpropionic acid, on the other hand, again yielded hippuric acid in the urine, thus showing that two atoms of the side chain were removed.

[15] C. P. Stewart, R. Gaddie, and D. M. Dunlop, Biochem. J. **25**, 733 (1931).
[16] I. Smedley-Maclean and M. S. B. Pearce, Biochem. J. **28**, 486 (1934).
[17] E. Knoop, Beitr. Chem. Physiol. Path. **6**, 150 (1904).

TABLE III

Acid Fed		Oxidation Product	Excreted as
Benzoic	C_6H_5COOH	Not oxidized	Hippuric
Phynylacetic	$C_6H_5CH_2COOH$	Not oxidized	Phenaceturic
Phenylpropionic	$C_6H_5CH_2CH_2COOH$	C_6H_5COOH	Hippuric
Phenylbutyric	$C_6H_5CH_2CH_2CH_2COOH$	$C_6H_5CH_2COOH$	Phenaceturic
Phenylvaleric	$C_6H_5CH_2CH_2CH_2CH_2COOH$	C_6H_5COOH	Hippuric

Other evidence is available. In diabetes, the oxidation of fat is incomplete, with the result that β-hydroxybutyric and acetoacetic acid are found in the urine. The formation of acetone bodies cannot be explained otherwise than by assuming that the long-chain fatty acids of an even number of carbon atoms are oxidized successively an even number of carbon atoms at a time. Also, the fact that the naturally occurring fatty acids almost invariably have an even number of carbon atoms indicates rather definitely that the synthesis and oxidation of the fatty acids involve an even number of carbon atoms.

Quick[18] states that for the short-chain fatty acids β-oxidation is the exclusive method by which fatty acids are oxidized.

Smedley-Maclean and Pearce[19] suggest that there are other modes of oxidation besides β-oxidation. They point out that all the feeding experiments have been conducted with fatty acids ten carbon atoms or less in length and, furthermore, the fatty acids had phenyl groups attached to one end which might materially affect the normal oxidation of the fatty acids.

Verkade and Van der Lee[02] fed various simple triglycerides to humans and analyzed the urine for decoic acids. The term ω-oxidation is applied to the following type of oxidation:

$$CH_3(CH_2)_9COOH \rightarrow HOOC(CH_2)_9COOH$$

They obtained the following results:

Tritridecylin.....	Not diacidogenic
Trilaurin.....	Practically not diacidogenic
Triundecylin.....	Strongly diacidogenic
Tricaprin.....	Strongly diacidogenic
Trinonylin.....	Rather weakly diacidogenic
Tricaprylin.....	Weakly diacidogenic

There is no contrast between the odd and even acids.

[18] A. J. Quick, J.B.C. 77, 581 (1928).
[19] I. Smedley-Maclean and M. S. B. Pearce, Biochem. J. 25, 1252 (1931).
[20] P. E. Verkade and J. Van der Lee, Biochem. J. 28, 31 (1934).

Flaschenträger and Bernhard[21] believe that they have definite proof of the occurrence of ω-oxidation. They believe, however, that it occurs to the extent of not more than 1 per cent of the total fatty acid oxidation and that the fundamental concept of β-oxidation in animal metabolism remains unshaken.

Butts *et al.*,[22] stimulated by the results of Clutterbuck and Raper, fed the sodium salts of propionic, butyric, β-hydroxybutyric, acetoacetic, valeric, caproic, heptoic, and caprylic acids to fasting female rats and determined the amount of acetone bodies in the urine. They found that butyric and caproic were ketogenic and that caprylic was twice as ketogenic as an isomolecular amount of diacetic acid, and they suggest that the only possible explanation is that the caprylic acid had undergone δ-oxidation. It was concluded that β- and δ-oxidation play important rôles.

Butts *et al.*[23] were able to show a marked difference between even- and odd-numbered carbon atom fatty acids. The odd-numbered acids gave rise to considerable glycogen formation in the liver whereas the even-numbered acids did not. They argue that the odd-numbered fatty acids are converted to propionic acid by β-oxidation, which in turn forms glycogen. The even-numbered acids, on the other hand, give rise to acetic acid by β-oxidation, and it has been shown that acetic acid does not give rise to glycogen.

Leathes suggests that the fatty acids are desaturated prior to burning. In this connection, however, Smith[24] reported that azelaic acid was utilized to only a very small extent by dogs. It is hard to see what virtue desaturation would have unless it gave rise to an acid which could be utilized.

It has been found by Mazza and associates that a Δ1,2 oleic acid was not dehydrogenated by either dead bacteria or liver extract, both of which are quite active upon stearic or ordinary Δ9,10 oleic. The Δ1,2 oleic was, however, vigorously oxidized by living bacteria and liver slices. It is argued that desaturation in the tissue involves the 1,2 position. Such desaturation could not be detected by ordinary iodine number determinations because the 1,2 position absorbs iodine to only a small extent.

Ketogenesis. Ketosis occurs in diabetes, in starvation, during the early stages of phosphorus poisoning, during anesthesia, in children during infection, on a high-fat diet, and in other conditions. The ketone

[21] B. Flaschenträger and K. Bernhard, Helv. Chim. Acta **18**, 962 (1935).
[22] J. S. Butts *et al.*, J.B.C. **109**, 597 (1935).
[23] H. J. Devel, J. S. Butts, L. F. Hallman, and C. H. Cutler, J.B.C. **112**, 15 (1935).
[24] H. G. Smith, J.B.C. **103**, 531 (1933).

or acetone bodies consist of β-hydroxybutyric acid, acetoacetic acid, and acetone.

The complete oxidation of fatty acids is in some way connected with carbohydrate metabolism. In diabetes, the failure to oxidize the fatty acids completely can be related directly to faulty carbohydrate metabolism. When carbohydrate utilization is improved, as by the administration of insulin, the formation of acetone bodies ceases. Similarly, during fasting as long as glucose is present, fat metabolism proceeds normally, but just as soon as the reserve carbohydrate is depleted and the starving animal has only fat and protein to draw on, the products of incomplete fatty acid oxidation appear. As indicated, protein as well as fat is capable of producing acetone bodies. The amino acids which yield acetone bodies are leucine, tyrosine, and phenylalanine.

Antiketogenesis. Shaffer[25] found that in order to get complete oxidation of acetic acid with hydrogen peroxide *in vitro* it was necessary to have one molecule of glucose to two molecules of fatty acid. These oxidations were carried out in an alkaline medium, and it was thought that a highly oxidizable compound was formed with acetoacetic acid and an intermediate product of glucose metabolism.

In a clinical study of the problem, Woodyatt[26] found that, for the complete oxidation of 1.5 grams of fatty acid, 1 gram of glucose must be utilized. This, on a molecular basis, would correspond to a ratio of about one. If this ratio is exceeded, ketosis appears. Since two of the acetone bodies are fairly strong acids, their appearance means an upset of the acid-base balance of the body. They are excreted partly in combination with fixed base and partly in combination with ammonia. This accounts for the depletion of the alkali reserve of the body and for the increased formation of ammonia in diabetics. This leads to a condition of acidosis and coma.

Macallum[27] has made some very interesting suggestions regarding the significance of ketosis. He points out that all the β-hydroxy and β-oxy acids are insoluble except β-hydroxybutyric and acetoacetic acid and that, owing to the solubility of these last two, they can diffuse out of the cell, where there is an adequate mechanism to oxidize them, into the blood stream where there is no method of disposing of them. Ordinarily, only a limited amount of fat is burned because glucose is oxidized more readily and this decreases the necessity or the possibility of extensive fat oxidation. When sugar cannot be oxidized, however, as in diabetes or during fasting when the carbohydrate stores have been

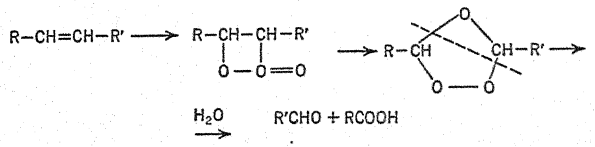
[25] P. A. Shaffer, Harvey Lectures **18**, 105 (1923).
[26] R. T. Woodyatt, Arch. Internal Med. **28**, 125 (1921).
[27] A. B. Macallum, Canadian Med. Assoc. J. **22**, 3 (1930).

depleted, the fat is called upon with a result that large quantities of the β-hydroxybutyric and acetoacetic acid are formed and pass into the blood. To a certain extent a fat tolerance can be built up so that larger and larger amounts of fat can be burned without the appearance of acetone bodies.

THE UNSATURATED FATTY ACIDS

The unsaturated fatty acids contain, for the most part, eighteen carbon atoms, though there are a number of exceptions to this rule. They are rather unstable, undergoing spontaneous oxidation and acquiring a brown coloration thereby. The structure of an unsaturated fatty acid can usually be determined by reducing the unsaturated fatty acid with hydrogen to the corresponding saturated acid whose constitution is known, or what is sometimes more convenient, to form the bromide of the saturated fatty acid; thus linolenic acid forms the hexabromide of stearic acid.

The position of the double bond can be determined by obtaining the ozonide, treating this with water, which splits according to the following reactions:



By a study of the fragments, the structure of the original molecule can be deduced. For example, ordinary oleic acid on such treatment yields three products: (1) nonyl aldehyde, ($CH_3(CH_2)_7CHO$); (2) nonylic acid, ($CH_3(CH_2)_7COOH$); and (3) azelaic acid, ($HOOC(CH_2)_7COOH$). These fragments show that in the original acid the double bond occurred between the ninth and tenth carbon atoms.

Isomerism. There are a number of isomers of oleic acid. Theoretically, at any rate, sixteen possible positions exist for the double bond, and several of these have been realized, as the following table shows.

TABLE IV

Acid	Melting point
Δ2,3 oleic	59°
Δ3,4 oleic	56°
Δ4,5 oleic	52°
Petroselenic acid, Δ6,7,	33°
Ordinary oleic, Δ9,10	15°
Isoleic Δ10,11	44°

In addition to this type, which is called position isomerism, another kind is encountered in the unsaturated fatty acids which is called geometrical isomerism. Here we have the *cis* and *trans* forms—terms which are familiar and scarcely need to be explained. Elaidic acid is the *trans* isomer and oleic is the *cis* isomer. Oleic can be transformed into elaidic acid by treatment with nitrous acid. Iwamota[28] reports that it is possible to change unsaturated *cis* fatty acids to the *trans* by means of silent electric discharge. As far as the author is aware, elaidic acid has no natural occurrence. But if one considers the elaidic acid isomers, there are an additional sixteen isomers of the single double bond, eighteen-carbon-atom fatty acid which gives, in all, thirty-two isomers of this acid.

Cooper and Edgar[29] point out that considerable biological significance attaches to the *cis-trans* isomerism. They found that, in general, the dibasic unsaturated *trans* acids are superior to the *cis* isomers in bactericidal action, although they are weaker acids. They are also more efficacious as protein precipitants and more reactive towards enzymes than the *cis* forms.

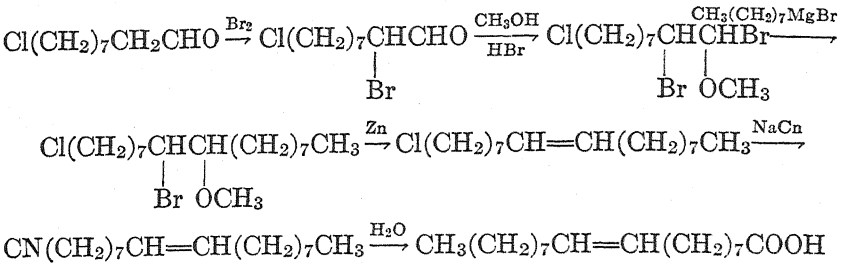
Reactions. Oleic acid goes through a number of typical reactions. On oxidation with alkaline potassium permanganate it yields dihydroxystearic acid. Halogens experience no difficulty in adding at the double bond, and, in fact, this is the basis for a quantitative estimation of the extent of unsaturation.

Preparation. Oleic acid has been highly purified by Lapworth, Pearson, and Mottram,[30] and its properties studied. They made the lead salt, which was purified. Then the acid was regenerated and fractionally distilled, and finally the barium salt was made and purified.

Brown and Shinowara[31] have recently investigated the possibility of preparing pure oleic acid by crystallization from acetone at $-60°$ C. The fatty acids from olive oil were dissolved in acetone and the saturated fatty acids allowed to crystallize at $-20°$ C. The temperature of the filtrate was lowered to $-60°$ C. and the precipitated oleic acid separated and recrystallized. The oleic acid, which was the $\Delta 9,10$ isomer, was reported to be very pure, with a sharp melting point of 13° C. The yield was 95 to 96 per cent of the theoretical.

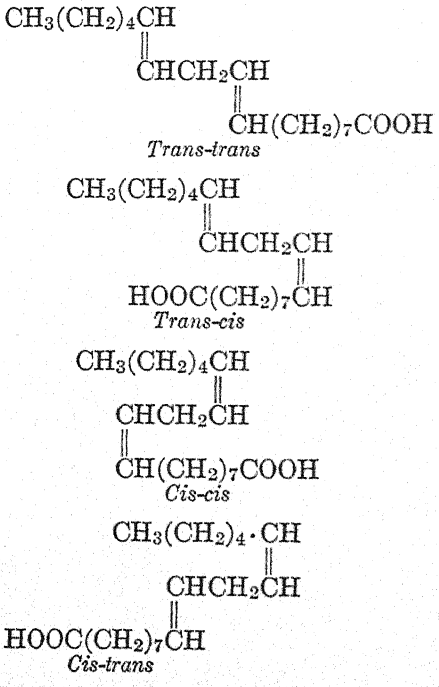
Synthesis. Noller and Bannerot[32] were able to synthesize oleic and elaidic acids according to the following reactions:

[28] Y. Iwamota, J. Chem. Soc. Japan **52**, 433 (1931).
[29] E. A. Cooper and S. H. Edgar, Biochem. J. **20**, 1060 (1926).
[30] A. Lapworth, L. K. Pearson, and E. N. Mottram, Biochem. J. **19**, 7 (1925).
[31] J. B. Brown and G. Y. Shinowara, private communication.
[32] C. R. Noller and R. A. Bannerot, J.A.C.S. **56**, 1563 (1934).



The synthetic mixture of acids contained approximately 63 per cent of elaidic acid, which is in fair agreement with the results of Griffiths and Hilditch,[33] who found from inversion experiments that the equilibrium mixture contains 34 per cent oleic and 66 per cent elaidic acid. Noller and Bannerot report satisfactory yields and suggest that the synthesis might be made a general one for unsaturated fatty acids.

Linoleic Series. The next more unsaturated fatty acid of the eighteen-carbon series is linoleic, which has two double bonds. Ordinary linoleic acid apparently has its unsaturation between the ninth and tenth and between the twelfth and thirteenth carbon atoms. With linoleic we have the possibility of four geometric isomers as follows:



[33] H. N. Griffiths and T. P. Hilditch, J. Chem. Soc. **1932**, 2315.

Of these, only the *trans-cis* and *trans-trans* have been positively identified. The tetrabromostearic acid is soluble in ether. Linoleic acid can be prepared from corn oil by the method of Rollett,[34] who brominated the hydrolyzed acids in ligroin and recrystallized the tetrabromide from ligroin. The tetabromide was then debrominated by refluxing in methyl alcohol to which zinc and HCl had been added.

Brown and Stoner[35] have recently studied the possibility of the preparation of linoleic acid by a direct crystallization of the acid at low temperatures in various solvents. They point out that, in Rollett's method, considerable quantity of the acid is converted into petroleum ether-soluble isomeric bromides and is lost and also that, owing to this alteration of the structure of the acid during bromination and debromination, the acid which is recovered may contain unknown amounts of isomeric forms. They found that crystallization of the fatty acids from methyl alcohol and acetone at $-20°$ C. gave separations of saturated and unsaturated acids at least as sharp as the standard lead soap-ether procedure; the saturated acids are insoluble in these solvents. The linoleic was partly separated from the oleic by lowering the temperature to $-50°$ C. or lower, whereupon oleic acid crystallized out. This separation is only partial, owing to the solubility of oleic acid in the solvents used (methyl alcohol, acetone, ethyl alcohol, and acetone-ethyl alcohol mixture). The highest purity of linoleic acid obtained was 92.7 per cent, using the fatty acids from corn oil and acetone at $-80°$ C., although in this case the yield was poor. They report satisfactory yields of linoleic acid with a purity of 87.7 per cent.

Fractional crystallization of the unsaturated methyl esters of the fatty acids from methyl alcohol at low temperatures was also studied and fair purity of acid obtained.

Linolenic Series. Linolenic acid can be prepared from linseed oil by the method of Rollett[36] through the hexabromide. The hexabromide is recrystallized until its melting point has risen to 180° C. It is then debrominated, esterified, and distilled at less than 1 mm. pressure. There is no definite configurational meaning in the terms α and β as they are commonly used in reference to the double- and triple-bond acids. The α-designation refers to those acids forming insoluble bromo addition compounds; the β refers to the isomers forming bromo addition compounds soluble with the solvents used.

Four linolenic acids have been partially identified. It was formerly thought that elaeostearic acid, the glyceride of which makes up about 90

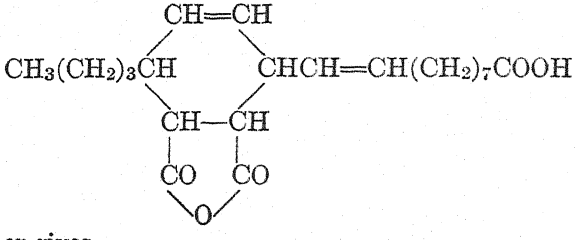
[34] A. Rollett, Z. physiol. Chem. **62**, 410 (1909).
[35] J. B. Brown and G. G. Stoner, private communication.
[36] A. Rollett, Z. physiol. Chem. **62**, 422 (1909).

per cent of tung oil, had two double bonds, but Boëseken and Ravenswaay,[37] through study of the iodine absorption for six days under the influence of ultra-violet light in conjunction with a knowledge of the index of refraction of the acid, came to the conclusion that the acid has three double bonds. This is in keeping with our knowledge of the drying properties of tung oil.

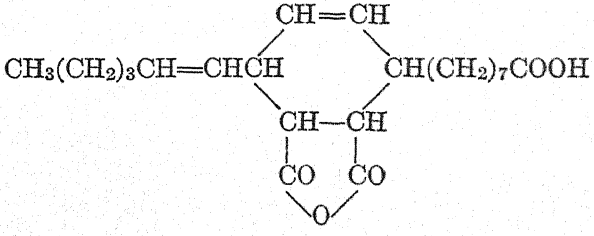
Probably the reason why iodine ordinarily adds at only two of the three double bonds of elaeostearic acid is that they occur as conjugated bonds. Elaeostearic acid has been assigned the following structure:[38]

$$CH_3(CH_2)_3CH : CHCH : CHCH : CH(CH_2)_7 \cdot COOH$$

Elaeostearic acid occurs as α- and β-isomers corresponding to the two α- and β-glycerides. There are eight possible *cis* : *trans* isomers of elaeostearic acid. Morrell and Samuels[39] found that the two isomers react with maleic anhydride. The α-isomer yields



while the β-isomer gives



Morrell and Davis,[40] on the basis of this evidence, concluded that the α-isomer probably has the *trans-cis-cis* and the β-isomer has the *cis-cis-trans* structure. Incidentally, only the maleic anhydride compound of the β-isomer and not those of the α-isomer will set to hard films. This suggests the importance of the double bond farthest from the carboxyl in the process of "drying."

[37] J. Boëseken and H. J. Ravenswaay, Chem. Abs. **19**, 2475.
[38] J. Boëseken, Analyst **54**, 305 (1929).
[39] R. S. Morrell and H. Samuels, J.C.S. **1932**, 2251.
[40] R. S. Morrell and W. R. Davis, Trans. Faraday Soc. **32**, 209 (1936).

Synthesis of the Unsaturated Fatty Acids. The unsaturated fatty acids may be synthesized in a number of ways. The unsaturated alcohol can be used as a starting point, Perkin's reaction can be employed, the unsaturated aldehyde can be oxidized, or certain lactone carbonic acids can be distilled and carbon dioxide split out. Actually, it is usually best to separate the unsaturated acid from natural oils.

Reference has already been made to the synthesis of oleic and elaidic acids.[32]

The production of unsaturated acids in nature is still being warmly debated. There is no question that desaturation can take place, but where it occurs or to what extent in animals is not definitely known.

The unsaturated acids are probably not synthesized as such directly from the carbohydrates, although the very wide occurrence of oleic acid and the fact that it is more abundant than stearic acid suggest that this acid may be directly synthesized.

The presence of dehydrogenases has been described in both plants and animals, but reports on dehydrogenases capable of activating the hydrogen of fatty acids are few. Tangl and Berend,[41] and later Berend,[42] claimed to have found a dehydrogenase in the pancreas which is capable of desaturating fatty acids to a considerable extent in a fairly short time. They even make the suggestion that the saturated fatty acids are desaturated in the intestines and are thus rendered diffusible and capable of being absorbed. The fact that unsaturated acids are found in nature is presumptive evidence for the occurrence of dehydrogenases.

Leathes[43] suggested that the liver is the center of fat metabolism and that the fatty acids are desaturated in the liver. He cites, in support of this view, (1) the high degree of unsaturation of the liver fatty acids. The iodine number of the liver fatty acids ordinarily range between 130 and 140, and the extent of unsaturation can be greatly increased by the feeding of some oil such as cod-liver oil. And (2) Hartley[44] found an oleic acid in the liver of the pig with its double bond between the twelfth and thirteenth carbon atoms and a linolenic acid with its double bonds between the ninth and tenth, and twelfth and thirteenth carbons. Neither of these acids is present in lard, the adipose tissue of the hog. It was argued, therefore, that desaturation must have taken place in the liver. Channon, Irving, and Smith[45] analyzed pig liver fat and were unable to find a trace of Hartley's $\Delta 12,13$ oleic

[41] H. Tangl and N. Berend, Biochem. Z. **220**, 234 (1930).
[42] N. Berend, Biochem. Z. **260**, 490 (1933).
[43] J. B. Leathes, Harvey Lectures, page 213, 1908.
[44] P. Hartley, J. Physiol. **36**, 17 (1907); **38**, 353 (1909).
[45] H. J Channon, E. Irving, and J. A. B. Smith, Biochem. J. **28**, 840 (1934).

acid. At least 85 per cent of the oleic acid was the Δ9,10 modification with possibly 10 per cent of the Δ10,11 isomer. Again, it is difficult to settle definitely whether the high degree of unsaturation of the liver lipids is due to simple selection on the part of the liver of the unsaturated acids or whether desaturation actually takes place. The evidence is inconclusive.

The recent work of Burr *et al.*, which will be referred to extensively later, seems to indicate that the rat, at any rate, is unable to synthesize linoleic and linolenic acids. On the other hand, arachidonic acid which, as will be recalled, is a C_{20} acid with four double bonds, occurs to a considerable extent in beef livers, and there are reports of its occurrence in other livers.[46] This acid does not occur in the diet and, therefore, must have been synthesized in the animal body. The state of the question at present, then, is that the animal body can synthesize some unsaturated fatty acids but the rat, and perhaps other animals also, is not able to synthesize linoleic or linolenic acids.

Position of Double Bonds. The unsaturated fatty acids show a tendency for the double bonds to occupy definite positions in the chains. The first double bond usually occurs on a multiple of three carbon atoms from the carboxyl, counting the carboxyl carbon as number one. The next double bond begins on a third carbon or a multiple of this number from the first double bond. This tendency towards regularity is shown in the following list of naturally occurring unsaturated fatty acids:

$C_{10}H_{18}O_2$	Deconoic	$CH_2{=}CH(CH_2)_7COOH$
$C_{14}H_{26}O_2$	Myristoleic	$CH_3(CH_2)_3CH{=}CH(CH_2)_7COOH$
$C_{16}H_{30}O_2$	Palmitoleic	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
$C_{18}H_{34}O_2$	Oleic	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
$C_{18}H_{34}O_3$	Ricinoleic	$CH_3(CH_2)_5CHOHCH_2CH{=}CH(CH_2)_7COOH$
$C_{18}H_{32}O_2$	Linoleic	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
$C_{18}H_{30}O_2$	Linolenic	$CH_3CH_2CH{=}CHCH_2CH{=}CHCH_2CH{=}CH(CH_2)_7COOH$
$C_{18}H_{34}O_2$	Petroselinic	$CH_3(CH_2)_{10}CH{=}CH(CH_2)_4COOH$
$C_{18}H_{32}O_2$	Tariric	$CH_3(CH_2)_{10}C{\equiv}C(CH_2)_4COOH$
$C_{18}H_{30}O_2$	Linolenic (*Oenothera*)	$CH_3(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)CH{=}CH(CH_2)_4COOH$
$C_{22}H_{42}O_2$	Erucic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{11}COOH$
$C_{22}H_{42}O_2$	Cetoleic	$CH_3(CH_2)_9CH{=}CH(CH_2)_9COOH$
$C_{24}H_{46}O_2$	Nervonic	$CH_3(CH_2)_7CH{=}CH(CH_2)_{13}COOH$

[46] W. R. Bloor and R. H. Snider, J.B.C. 87, 399 (1930); 99, 555 (1933).

The possibility of isomerism in the unsaturated acids is very large. For example, if both the geometrical and position isomers are considered, thirty-two oleic acids are possible, something over three hundred and sixty linoleics, and a correspondingly greater number of linolenics.

THE ESSENTIAL FATTY ACIDS

In 1927, Evans and Burr[47] published the results of experiments with highly purified diets. It was shown that low-fat diets markedly retarded the growth and sexual maturity of rats. Ovulation and reproduction almost completely failed on the low-fat diets. The next year, McAmis, Anderson, and Mendel[48] reported subnormal weight for rats on a low-fat diet, and Burr and Burr[49] found that a definite deficiency disease resulted from the rigid exclusion of fat from the diet. Subsequent papers by Burr and co-workers[50, 51] showed that the curative value of oils depends upon the degree of unsaturation, and that of the known fatty acids only linoleic and linolenic are highly efficacious as preventives or curatives. These two fatty acids are now called "essential fatty acids." Either one will suffice. Brown and Burr have recently pointed out, however, that tissue normally has little linolenic acid (it may often be undetectable) whereas linoleic acid is a fairly constant constituent of the essential phospholipids and is the one which most likely is chiefly involved in the low-fat deficiencies. Evidence is still lacking concerning the dietary requirements of the more unsaturated, long-chain acids such as arachidonic and clapanodonic.

The effects of extreme fat deficiency are severe. A rat ceases growing when it has reached about 75 per cent of the normal adult weight and dies when it has lived about one-third of its normal life span. Among the early abnormalities may be found scaly skin, high water consumption, abnormal metabolism, delayed sexual development. Later, hematuria frequently occurs; reproduction either fails or is abnormal; lactation is deficient.

The reproduction failure[52] observed in fat-deficient animals is apparently due to changes in the uterine wall which interfere with the nutrition of the fertilized ovum. If implantation takes place, fetal death may occur at any time during pregnancy and is apparently caused

[47] H. M. Evans and Geo. O. Burr, Proc. Soc. Exptl. Biol. Med. **25**, 41 (1927).
[48] A. J. McAmis, W. E. Anderson, and L. B. Mendel, J.B.C. **82**, 247 (1929).
[49] Geo. O. Burr and M. M. Burr, J.B.C. **82**, 345 (1929).
[50] Geo. O. Burr, M. M. Burr, and E. S. Miller, J.B.C. **97**, 1 (1932).
[51] Geo. O. Burr and W. R. Brown, Soc. Exptl. Biol. Med. **28**, 905 (1931); **30**, 1349 (1933).
[52] Ed. C. Maeder, thesis in progress, Department of Obstetrics and Gynecology, University of Minnesota.

by placental injury. Degeneration changes in the ovaries sometimes occur. Reproduction does not become normal until some time after clinical cures have been obtained.

A respiratory quotient greater than unity indicates the transformation of carbohydrates to fat. One peculiarity of the fat-deficiency disease is that such a respiratory quotient is obtained a few hours after feeding, indicating a synthesis of fat, but the rat continues to suffer from a lack of the essential fatty acids.

Very small amounts of the unsaturated fatty acids are effective in curing the deficiency disease. One-half drop of methyl linoleate daily will cause the scaliness of feet, tail, and skin to disappear and will restore growth to a fat-deficient rat. Such a small dose, however, will not allow satisfactory gestation and lactation. A 3 per cent level of lard (one of the best nutritional fats) will permit normal growth and gestation, but the level is still too low for satisfactory lactation.[53]

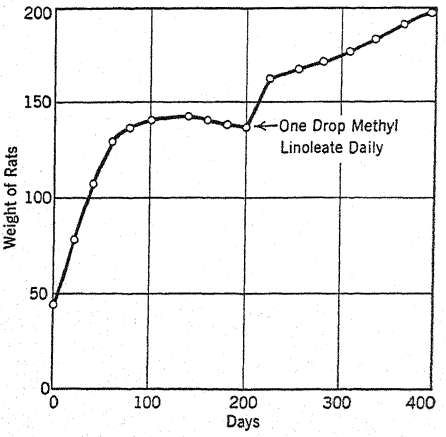


FIG. 3.—Curative effect of methyl linoleate.

Fig. 3, taken from the work of Burr and co-workers, shows the spectacular response in growth of rats to methyl linoleate.

Many substances were tested for their curative properties. Oleic, α-elaeostearic acids were ineffective. Fig. 4 shows the comparison of lard, hydrogenated cocoanut oil, and yeast oil.[51]

Burr and Brown are now studying the milk lipids. Their results so far indicate that sucklings of carnivorous and omnivorous animals obtain fairly large amounts of unsaturated fatty acids. The milk of herbivorous animals apparently has less unsaturated fatty acids than the other groups.

Although no pathological symptoms appeared in an adult on a fat-free diet over a period of six months, the metabolic studies strongly indicate that, on very low fat diets, human beings may be expected to react in a manner similar to rats.

The results of Burr and co-workers have been criticized by other workers. The essentials appear to have been confirmed, however. The

[53] G. O. Burr *et al.*, private communication.

principal criticism seems to be that the skin effects are variable and may be produced by other dietary deficiencies. Recently, Brown and Burr[54] have found that the scaly condition of the feet and tails of rats is decreased by increasing humidity and offer this suggestion as a reason

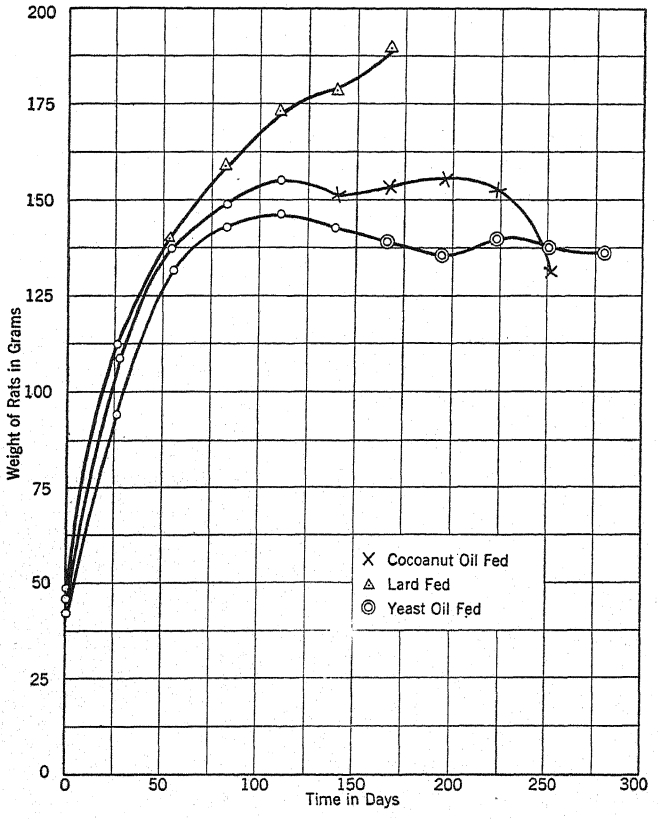


FIG. 4.—Comparison of the curative effects of lard, hydrogenated cocoanut oil, and yeast oil.

why workers in some localities fail to obtain the skin symptoms on a fat-deficient diet.

Recently, Sinclair[55] fed rats casein, salt mixture, yeast, and elaiden (74 per cent calories), supplemented with cod-liver oil concentrate, and obtained cessation of growth when a weight of 80 to 90 grams had been attained. If sucrose then replaced the elaiden, growth was resumed at

[54] W. R. Brown and Geo. O. Burr, J.B.C. **114**, I (1936).
[55] R. G. Sinclair, J.B.C. **114**. xciv (1936).

the rate of about 20 grams per week until the rats eventually reached the same weight as rats fed from weaning age on the same fat-free diet. Sinclair interprets this evidence to indicate a limited synthesis of the higher unsaturated fatty acids from carbohydrates which is almost completely abolished by the high-elaiden diet.

The work of Burr *et al.* is of significance in relation to the ability of the animal body to desaturate fatty acids. His work seems to indicate the lack of such an ability. It may well be, however, that such inability applies to some particular isomer of linoleic or linolenic acids. When one remembers the enormous number of possible isomers of these acids, this does not appear improbable.

During a study of the problem of infantile eczema, Hansen observed that favorable clinical results were obtained in several instances when olive oil and corn oil were added to the diet. This observation led to a study of the blood lipids in this condition. Preliminary findings revealed that the serum of eczematous infants absorbed 383 mg. I_2 per 100 cc., whereas the serum from normal infants of a similar age absorbed 539 mg. I_2 per 100 cc. More extensive studies including eczema in older children as well as subjects with other pathological conditions resulted in the following findings:

TABLE V

	I_2 Absorbed Mg. 100 cc. Serum	Cholesterol Mg. Per Cent	Total Fatty Acid Mg. Per cent	I_2 No. Total Fatty Acid
Normal under 2 years.....	496	176	339	112
Exzema under 2 years.....	446	164	387	87
Normal over 2 years.....	565	199	359	121
Exzema over 2 years.....	498	190	368	102
Miscellaneous diseases....	627	231	390	123

The differences in the iodine number of the fatty acids were found to be statistically significant. This finding was confirmed by Faber and Roberts. This low degree of unsaturation of the blood lipids was also found by the microgravimetric technique of Wilson and Hansen, wherein the fatty acids are isolated and their qualitative characteristics obtained. The serum fatty acids from eczematous infants were found to have an iodine number of 88 with 0.97 double bonds per molecule of fat, while in normal control infants, the iodine number was 108 with 1.25 double bonds per molecule. On the analysis of larger samples of serum obtained by pooling specimens from eczematous subjects and from normal infants, Brown and Hansen found the linoleic acid (tetra-

bromides) and arachidonic acid (polybromides calculated as such) content to be lower in the serum from the patients with eczema.

Hansen further observed that the abnormally low iodine numbers of the serum fatty acids tended to rise to essentially normal levels at the time of the clinical improvement following the administration of such oils as raw linseed oil and corn oil to certain eczematous subjects. More evidence that the unsaturated fatty acids were important in eczema was suggested by the observation that the serum fatty acid iodine numbers were found to be higher at the time of clinical improvement following the local application of ointment containing crude coal tar. This type of therapy is ordinarily considered the most effective of the numerous remedies used, yet the mechanism is little understood. As pointed out by Hansen, further study is necessary to determine the limitations and indications of the clinical effectiveness, in cases of eczema, of the internal administration of oils rich in the unsaturated fatty acids. However, Cornbleet, as well as other workers, has been able to confirm these findings.

No extensive studies have been made on the effect of fat-free diets in children although a few isolated reports indicate that certain infants tend to develop eczematous manifestations under these conditions. Brown, Hansen, Burr, and McQuarrie found that a normal, healthy adult failed to develop any pathological symptoms when on a strictly fat-free regimen for a period of nearly six months. Metabolic studies indicate that the human being under these conditions behaves in a similar manner to the rat when on this type of diet. Blood lipid determinations showed that the degree of unsaturation of the serum fatty acids was definitely decreased under these conditions. Hansen and Burr[56] were the first to show that the iodine numbers of the serum lipids were low on a fat-free diet. The iodine numbers of the total lipids were found to be 118 in normal adult rats on stock diet in contrast to 90 in rats on the fat-deficient diet. This observation has been confirmed by Williams and Maynard in goats, also by Hansen, Wilson, and Williams in dogs. Hansen and Brown have shown that the iodine number of the serum lipids tends to vary directly with the iodine number of the fat in the diet, much in the same manner as reported by Anderson and Mendel for the body fats.

PHYSICAL PROPERTIES OF THE FATTY ACIDS

Dissociation Constants. The fatty acids are all weak acids, and their dissociation constants lie between 1 and 2×10^{-5},[57] as shown in Fig. 5.

[56] A. E. Hansen and G. O. Burr, Proc. Soc. Exptl. Biol. and Med. **30**, 1201 (1933).
[57] International Critical Tables 6.

There seems to be no consistent trend in the dissociation constants, and the observed variation is possibly due to errors in determination. The higher fatty acids being insoluble in water, no information concerning their dissociation constants in water is available. Fischgold and Chain[58] titrated stearic acid in 90 per cent ethyl alcohol and found it to have a *p*Ka of 7.3. This high value is probably related to the low dielectric constant of ethyl alcohol. The dissociation constants of the higher fatty acids, owing to their insolubility, have little meaning except where they occur in surfaces.

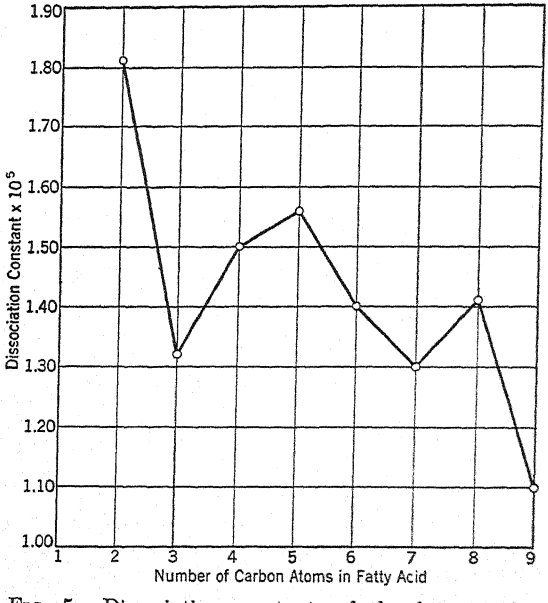


FIG. 5.—Dissociation constants of the fatty acids.

Myers,[59] from a study of the collapse pressure of monomolecular films of palmitic acid upon acid solutions which yield an S-shaped curve when collapse pressure of a film is plotted against *p*H, found the midpoint of the curve, which corresponds to the half neutralization, to be at *p*H3. This would indicate that the higher fatty acids occur at biological surfaces, where the neutral point is about *p*H7, largely in the form of soaps. This question has been investigated by Langmuir and Schaefer[60] for barium and calcium soaps of stearic acid and will be referred to under soaps.

Substitution of a halogen into a fatty acid or a change of its structure has a considerable effect on the dissociation constant of the acid, as the following data show:

CH_3COOH	1.86×10^{-5}
$CH_2ClCOOH$	1.55×10^{-3}
$CHCl_2COOH$	5.0×10^{-2}
CCl_3COOH	2.0×10^{-1}

[58] H. Fischgold and E. Chain, Biochem. J. **28**, 2044 (1934).
[59] R. J. Myers, J.A.C.S. **57**, 2734 (1935).
[60] I. Langmuir and V. J. Schaefer, J.A.C.S. **58**, 284 (1936).

N-Valeric	1.61×10^{-5}
iso-Valeric	1.73×10^{-5}
α-Chloropropionic	1.47×10^{-3}
β-Chloropropionic	8.59×10^{-5}

Langmuir[61] points out that the substituted chlorine has a greater tendency to take on electrons than the hydrogen atom which it displaces and, consequently, the electrons in the carboxyl group tend to shift in the direction of the chlorine atom. This shift of the electrons towards the chlorine atom makes it easier for the positive hydrogen, which is bound to the carboxyl group, to pass into the water to form the hydronium ion $(H_3O)^+$ with a resulting augmentation in the dissociation constant.

Crystal Structure. The physical constants of the fatty acids vary in a highly characteristic manner. Fig. 6 is a plot of the melting and boiling points of the fatty acids. It is seen that the melting points of the fatty acids give a step-like curve whereas that of the boiling points is smooth. The step-like curve of the melting points has its origin in the difference in crystal structure of the odd- and even-numbered fatty acids. It is apparently true that when some physical measurement yields alternating values between even and odd fatty acids we have good reason to believe that we are dealing with crystalline material.[62]

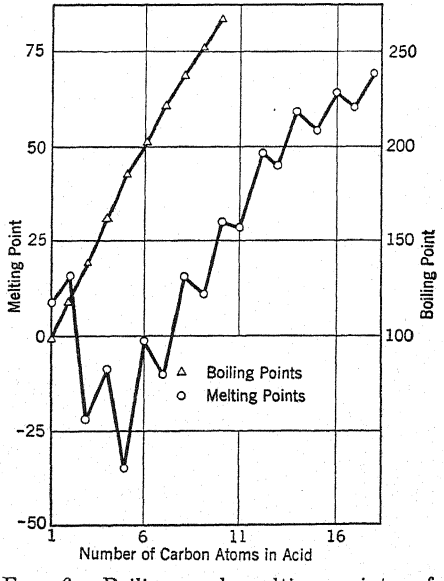


FIG. 6.—Boiling and melting points of the fatty acids.

Garner and King[63] studied the heats of crystallization of the even and odd series and found definite alternations. They also found evidence for at least two crystal forms in both of the series. The α-form appears when an acid solidifies and changes into the stable or β-form when the temperature is lowered a few degrees under the solidifying

[61] I. Langmuir, Chem. Rev. **6**, 465 (1929).
[62] O. G. Jensen and R. A. Gortner, J. Phys. Chem. **36**, 3138 (1932).
[63] W. E. Garner and A. M. King, J.C.S. **1929**, 1849.

temperature. The β form will not change into the α form if the temperature be raised again, without melting first. This one-way "equilibrium" is observed with acids having more than eleven carbon atoms; however, this is not true of the acids having eleven carbon atoms or less.

X-ray Analysis. The X-ray has been especially useful in clearing up the complexity of the crystal structure of the fatty acids. Several

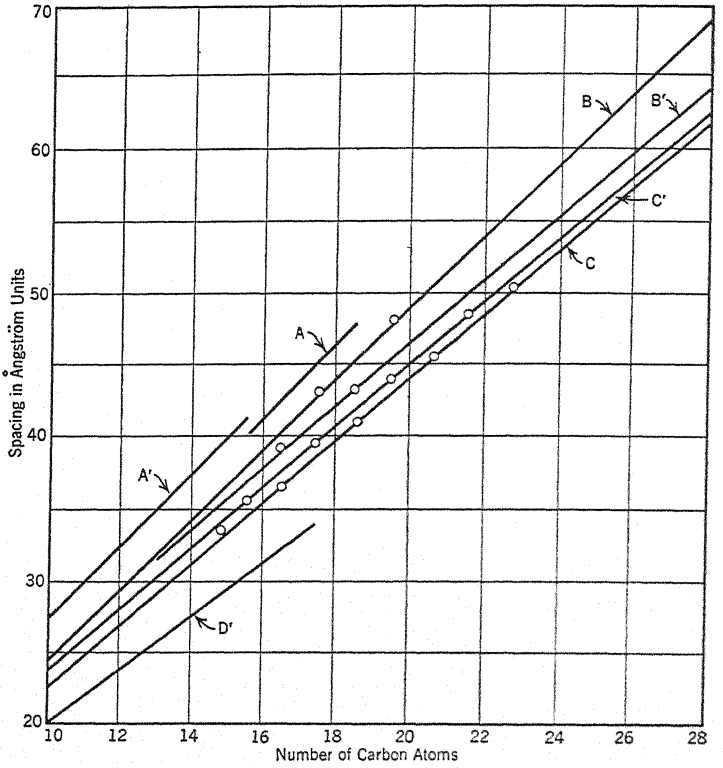


FIG. 7.—Long spacings of fatty acids as shown by X-ray.

papers on this subject have appeared, but we will refer to only two which are especially useful.[64, 65]

The fatty acids show three spacings. One is long and dependent on the number of carbon atoms; the other two are small, almost equal, and nearly independent of the carbon number. It is evident that the long spacing corresponds to approximately two molecular lengths of fatty acid. Fig. 7 is that given by Piper, showing the long spacings in Ångström units plotted against the carbon number.

[64] S. H. Piper, Trans. Faraday Soc. 25, 348 (1929).
[65] F. Francis, S. H. Piper, and T. Malkin, Proc. Roy. Soc. A128, 214 (1930).

The letters marked with a prime refer to the odd fatty acids. The C' and B', and C and B seem to be the most characteristic spacings. The C spacings are obtained with rapidly solidified fatty acid near its melting point (α-form of Garner and King). The B spacings are obtained with acids which have been allowed to crystallize slowly from a solvent (β-form of Garner and King?). C changes into B as the temperature is lowered. Piper finds it difficult or impossible to change B into C without first melting. The occasion for the A, A', and D' spacings is unknown. The fatty acids crystallize in layers with the carboxyl groups together and methyl groups together. This, indeed, is the reason for the double value of the molecular length of the long spacing.

Since the space occupied by a carbon atom is known from other sources, it is possible to calculate the angle of inclination of the fatty acids to the planes of cleavage. The C, B', and C' spacings all give an angle of inclination of 59° 12′. Other measurable slopes are B 74° 36′, A' 90°, and D' 52° 29′.

The intercepts of any one of the lines in Fig. 7 on the spacing axis gives the effective space occupied by terminal groups for that particular form, and it may be concluded that the crystal forms giving the spacings of B', C, and C' probably have similarly orientated chains but with different packings of the end groups.

Fig. 8 is a diagrammatic representation of two crystals of an even fatty acid. One is of the unstable (C) form and the other of the stable (B) form.

Francis *et al.*[65] have also studied the melting points and X-ray patterns of mixtures of fatty acids and of mixtures of ethyl esters. There is no alternation in the melting points of the ethyl esters. They observed that, with odd-numbered fatty acids, a definite change takes place when the material is cooled to about 10° C. below the solidifying point, the acid suddenly shrinking away from the glass of the tube with which it is in contact. This change is accompanied by a parallel change in the X-ray pattern.

All equimolecular mixtures of neighboring fatty acids showed as sharp melting points as the pure acid, and as in these, resolidification took place within one degree of the melting point. Equimolecular mixtures of the ethyl esters show spacings corresponding to a chain containing two carbon atoms more than the longer constituent. They used X-ray and melting-point technique to analyze mixtures of the higher fatty acids and applied this method to the analysis of some acids which are usually assumed to be pure, thus, a sample of so-called pure arachidic acid, which is the normal C_{20} acid, turned out to be a mixture of C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, and C_{26} acids. The sample of lignoceric acid

was found to contain only C_{24} acid. Cerotic acid contained some C_{28}. They[66] have since applied this method of analysis to the acid isolated from cerebron. Levene claimed this to be a hydroxy C_{25} acid, whereas Klenk thought it a hydroxy C_{24} acid. Chibnall, Piper, and Williams came to the conclusion that cerebronic acid is a mixture of C_{22}, C_{24}, and C_{26} α-hydroxy acids. They also analyzed the fatty acid from kerasin

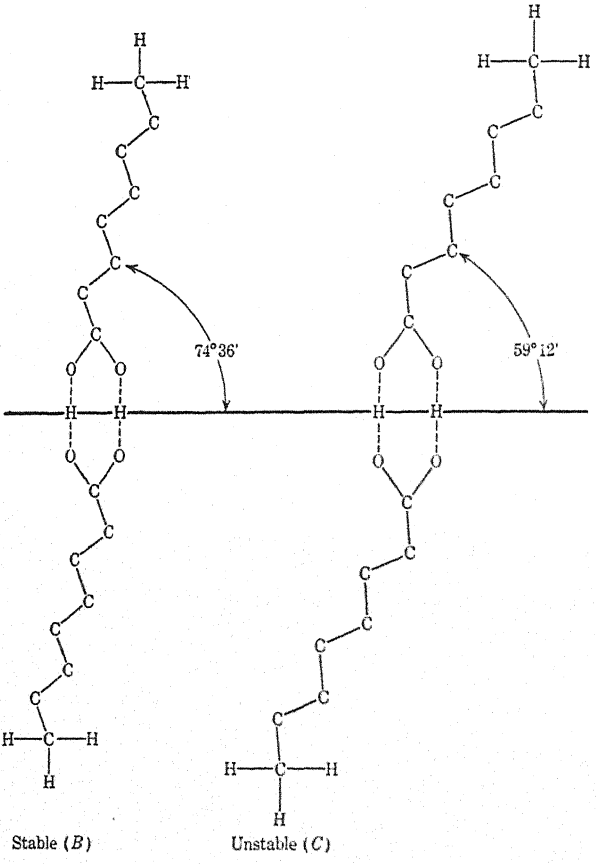


FIG. 8.—Diagrammatic representation of two crystals of an even-numbered carbon atom fatty acid.

and found it to be a mixture of C_{22}, C_{24}, and C_{26} saturated acids though formerly it was supposed to be lignoceric acid.

Fig. 9 shows the melting points of mixtures of some of the higher fatty acids.[66, 67]

[66] A. C. Chibnall, S. H. Piper, and E. F. Williams, Biochem. J. **30**, 100 (1936).
[67] S. H. Piper, A. C. Chibnall, and E. F. Williams, Biochem. J. **28**, 2175 (1934).

Molecular Orientation. Probably no other one concept has colored the science of chemistry in recent years to the extent that molecular orientation has. Credit for the first exposition of this concept must go to Hardy.[68] Langmuir[69] was able to demonstrate molecular orientation at an air-water interface in a very graphic manner. He showed that the higher insoluble fatty acids were orientated with their carboxyl groups sticking in the water and at high dilutions the paraffin chains floated horizontally on the surface. As these acid molecules were crowded together by diminishing the surface with a barrier, they stood on their heads, and at this point the area is constant until the film is ruptured. In Fig. 10 the force in dynes on the film of palmitic acid is plotted against the area per molecule in square Ångström units.

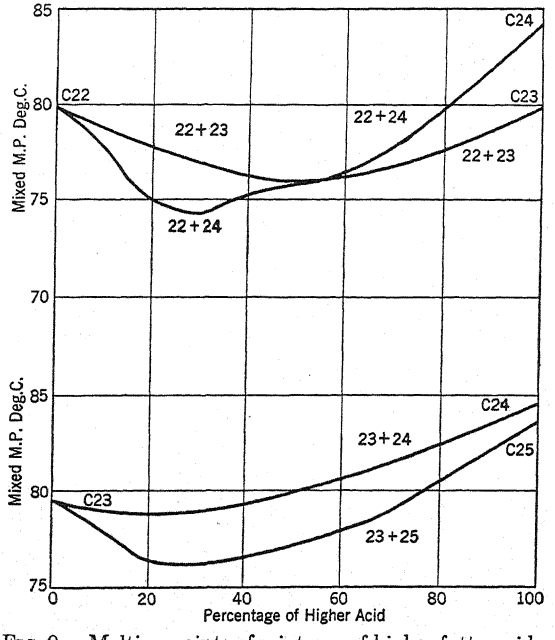


FIG. 9.—Melting points of mixtures of higher fatty acids.

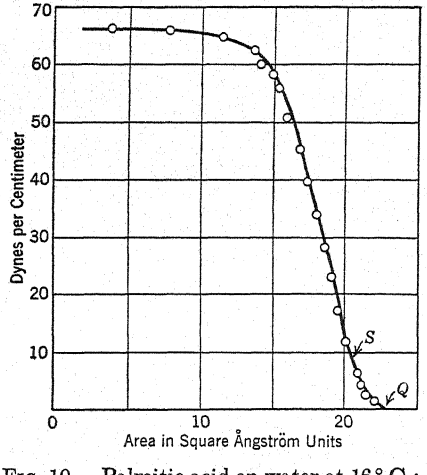


FIG. 10.—Palmitic acid on water at 16° C.; at *S* the film became solid.

Some substances, notably the ethyl esters of a dibasic acid, were found to give a gaseous film to which the gas laws relating temperature, pressure, and area could be applied. If Van der Waals' corrections were used, better agreement between theory and experiment was found.

[68] W. B. Hardy, Proc. Roy. Soc. **A88**, 303 (1913).
[69] I. Langmuir, J.A.C.S. **39**, 1848 (1917).

This type of orientation is due to the tendency of one part of the molecule to dissolve in water while the other part, *i.e.*, the paraffin chain, is quite insoluble in water. Certain groups tend to render an organic molecule soluble in water. These groups are called polar groups and are the NH_2, NO_2, SO_3H, OH, CN, COOH, CHO, the halogens, and ethylene bond. This is one concept of polarity.

Electrical dissymmetry in a molecule is another kind of polarity, and the dipole moment is a measure of it. The dipole moment is equal to the distance separating the charges in a molecule multiplied by the charge. Wilson and Wenzke[70] in a recent paper have studied the dipole moment of certain fatty acids in dioxan. Table VI shows their results.

TABLE VI

DIPOLE MOMENT OF FATTY ACIDS

Fatty Acid	Dipole Moment $\times 10^{18}$
Formic	2.07
Acetic	1.74
Propionic	1.75
Stearic	1.74

It can be seen that the dipole moments of the fatty acid do not vary with the carbon number. Incidentally, formic acid does not fit into the series. It should not be considered a fatty acid.

Capillary Activity and Adsorption. Certain substances decrease the surface tension of a liquid when they are added to the liquid. The more they decrease the surface tension, the more they are adsorbed. This relation is mathematically expressed by Gibbs' adsorption equation. The fatty acids, relatively speaking, are very capillary-active, *i.e.*, they reduce the surface tension of water to a large extent and are accordingly greatly adsorbed at the surface.

Fig. 11 shows the surface tension of an aqueous solution of several fatty acids plotted against the mol concentration of these acids.

It will be seen that the capillary activity of the acids increases geometrically with the length of the carbon chain. The geometrical ratio of the capillary activity between a fatty acid and its next lower homologue is approximately equal to 3.0. This is a statement of Traube's rule.

Langmuir[71] has discussed the theoretical aspects of Traube's rule.

[70] C. J. Wilson and H. H. Wenzke, J. Chem. Phys. 2, 546 (1934).
[71] I. Langmuir, J.A.C.S. 39, 1883 (1917).

Langmuir interprets the rule to mean that between two consecutive members of a homologous series there is a constant difference in the work required to take a mol of capillary-active material out of the surface into the liquid. For the fatty acids, this amounts to about 700 gram calories.

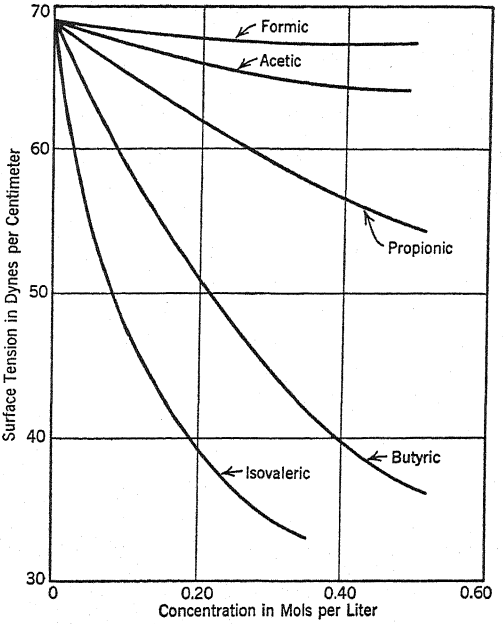


FIG. 11.—Surface tension of aqueous solutions of fatty acids.

A great deal of work has been done on the adsorption of fatty acids out of solution onto a solid phase. In most of these studies, some form of carbon has been used as the adsorbent. It has often been found possible to express the results of such a study by means of certain equations. An empirical equation which is often used is the Freundlich adsorption isotherm

$$\frac{a}{m} = \alpha C^{1/n}$$

where a is the amount adsorbed; C, the equilibrium concentration; m, the grams of adsorbent; and α- and $1/n$, empirical constants.

Langmuir[72] was able to derive an adsorption equation from theoretical considerations. It takes the form

$$a = \frac{\alpha\beta C}{I + \beta C}$$

where α and β are new constants with some theoretical significance.

Adsorption of fatty acids from aqueous solution follows a Traube's rule especially if the amount adsorbed is expressed in grams and not in mols. From time to time, reversals of Traube's rule has been reported. For example, Holmes and McKelvey[73] found such a reversal with the

[72] I. Langmuir, J.A.C.S. **40**, 1361 (1918).
[73] H. N. Holmes and J. B. McKelvey, J. Phys. Chem. **32**, 1522 (1928).

adsorption of fatty acid on SiO_2 in a non-polar solvent. Reversal has also been reported on certain charcoals, activated by heating under specified conditions.[74] By a reversal is meant a greater adsorption of the lower fatty acids than of the higher homologues. The only way to explain such a reversal, as far as the author is aware, is to assume that, in such cases, the fatty acids lie flat on certain carbon surfaces with the activated and now more polar carbon attracting the carboxyl group.

Linner and Gortner[75] made an interesting study of the adsorption of the fatty as well as the dicarboxylic acids on charcoal. They found that when β of the Langmuir adsorption isotherm (which is really proportional to the reciprocal of the surface occupied by the adsorbed acids) is plotted against the carbon number a step-like curve is obtained which resembles, in a marked fashion, that obtained from a plot of the melting point against the carbon number. The acids are apparently in a crystalline state on the surface.

Recently, Blodgett[76] has been able to deposit, on glass slides, polymolecular films of calcium palmitate, stearate, or arachidate, of almost any desired number of molecular layers. Two series of films are obtained, depending on the *p*H of the underlying solution. The *Y* films which are deposited when the glass slide is lowered or raised through the surface on which the fatty acid is spread have the carboxyl groups together and the methyl groups likewise together, as in a crystal of stearic acid. The *Y* layers are deposited at a *p*H of about 6.4. The *x* layers are deposited at a *p*H of 9 or above and only when the slide is lowered through the surface. Beautiful interference colors may be developed by the layers if the index of refraction of the glass slide is sufficiently different from that of the fatty acid (1.43). Ordinary microscope slides will not serve this purpose since the index of refraction of such slides is too close to that of the fatty acids. Special slides of thin optical glass serve admirably.

[74] B. Nekrason, Z. physik. Chem. **136**, 379 (1928).
[75] E. Linner and R. A. Gortner, J. Phys. Chem. **39**, 35 (1935).
[76] K. B. Blodgett, J.A.C.S. **57**, 1007 (1935).

CHAPTER II

THE SOAPS

Metals will replace hydrogen from the carboxyl group of a fatty acid to form a soap. These are familiar but complicated substances.

It is not easy to determine the degree of biological importance of soaps. There is reason to believe that if free fatty acid occurs in tissue, and if it is at an interface, a large fraction of it must be in the form of a soap at *p*H 7.3. Langmuir and Schaefer[1] analyzed the monomolecular film of stearic acid which was formed on barium and calcium bicarbonate solutions, and their results are shown in Fig. 12.

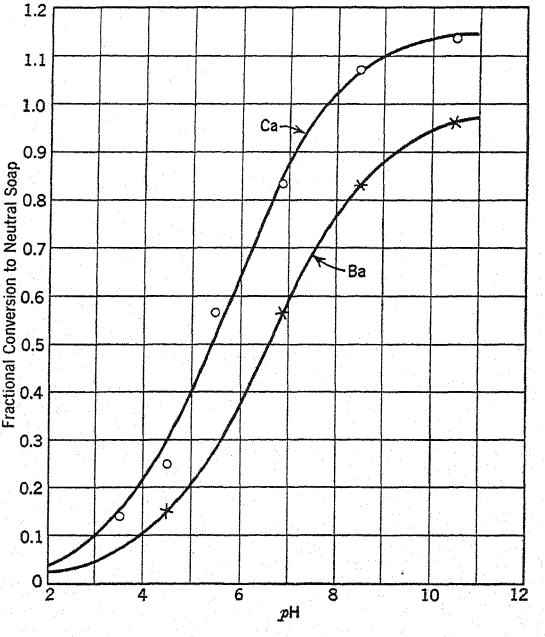


FIG. 12.—Fractional conversion of fatty acid to neutral soap as a function of *p*H.

It can be seen that at *p*H 7 an appreciable fraction of the stearic acid occurs as soap.

Powney[2] has investigated the effect of *p*H on the surface tension of certain soap solutions, and Fig. 13 shows some of his results.

The alkali metal, as well as the ammonium soaps, are very capillary-active and are good emulsifying agents, to which facts they no doubt owe most of their detergent properties.

Kneen and Benton,[3] using an apparatus which they developed, have

[1] I. Langmuir and V. J. Schaefer, J.A.C.S. **58**, 284 (1936).
[2] J. Powney, Trans. Faraday Soc. **31**, 1510 (1935).
[3] Eric Kneen and W. W. Benton, private communication.

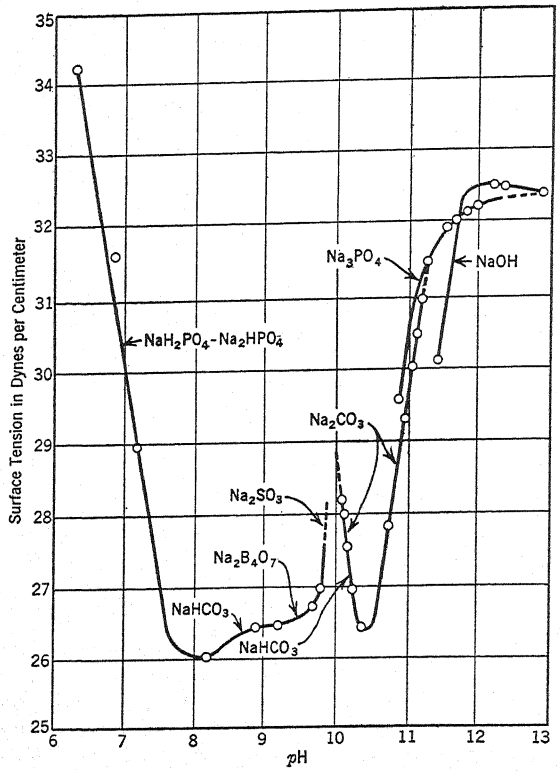


FIG. 13.—Surface tension—*p*H curve for 0.1 per cent sodium oleate

measured the contact angle between 0.01 *N* solutions of the sodium salts of some of the fatty acids and a solid paraffin surface. Zero contact angle indicates complete wetting, whereas an angle of 180° would indicate a complete absence of any tendency to wet. Fig. 14 shows their results.

This demonstrates in a very clear fashion the sharp break in properties with caprylic acid; it is at this point that the sodium salts take on soap properties. It was not possible for Kneen and Benton to determine the contact angle of the

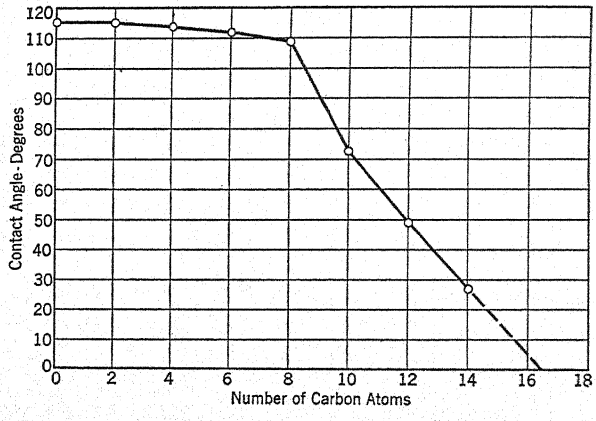


FIG. 14.—Contact angle of soap solutions on paraffin.

palmitate and stearate at the temperature at which they were working (25° C.) because these soaps do not form clear solutions at such a temperature. The straight line has, however, been extrapolated to the palmitate which yields a zero contact angle and indicates that the paraffin would be completely wet by the palmitate. The soaps, in order to clean a surface, must first wet it.

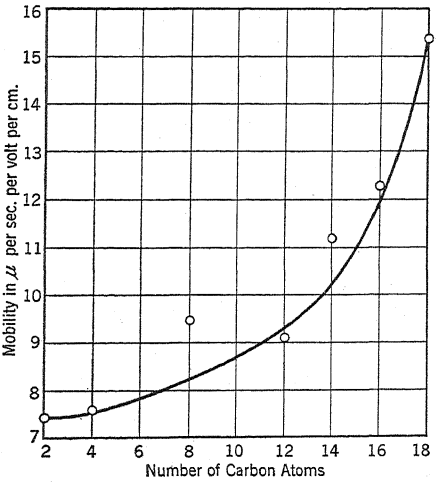


FIG. 15.—Electrokinetic potential of carbon particles in soap solutions.

Work of Urbain and Jensen[4] indicates that the soaps peptize dirt particles by imparting to them a higher electric surface potential (ζ-potential). They obtained the mobilities of carbon particles at 75° C. in 0.0036 *M* sodium soap solutions as shown in Fig. 15.

McBain has published a large number of papers dealing with soaps; they can be found for the most part in the Journal of the Chemical

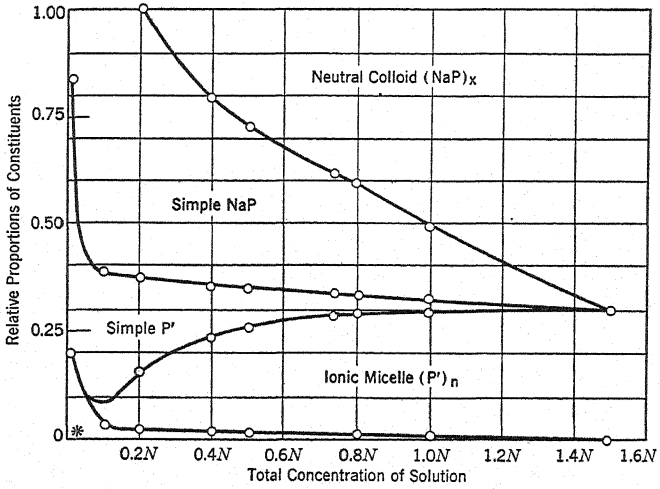


FIG. 16.—Composition of sodium palmitate solution as a function of concentration; * acid soap

[4] W. M. Urbain and L. P. Jensen, J. Phys. Chem., 40, 821 (1936).

Society of London. Fig. 16 illustrates the extreme complexity of a soap solution.[5]

The soaps of polyvalent metals are quite insoluble in water. These soaps act as water-in-oil emulsifiers in contrast to the soaps of the alkali metals, which are good oil-in-water emulsifiers. This difference in properties is reflected in the interfacial tension between paraffin oil containing 0.001 *N* oleic acid and aqueous solutions of varying concentrations of NaOH, NaCl, and $CaCl_2$, as shown in Table VII.[6]

TABLE VII

INTERFACIAL TENSION BETWEEN PARAFFIN OIL CONTAINING 0.001 *N* OLEIC ACID AND AQUEOUS SOLUTIONS OF NaOH, NaCl, AND $CaCl_2$

Salt Concentrations in Molarity			Interfacial Tensions in
NaOH	NaCl	$CaCl_2$	Dynes per Centimeter
0.001	0.0	0.0	7.2
0.001	0.15	0.0	0.0
0.001	0.0	0.0015	9.65
0.001	0.15	0.0015	7.48
0.001	0.30	0.003	7.12
0.001	0.45	0.005	7.36
0.001	0.60	0.01	8.20
0.0	0.0	0.0	31.05

There appears to be an antagonism between sodium and calcium. It has been suggested that this is the basis for ion antagonism so generally observed in living organisms.

Germicidal and Detoxifying Properties of Soaps. Several times prior to 1911, various workers had observed that ground tissue and tissue extracts, as liver, brain, etc., had germicidal properties. These were shown to be due to their lipid content.

Lamar[7] observed that a pneumococcus antiserum produced lysis of the cocci in the presence of soap. Ordinarily antiserum causes an agglutination of the organisms. Normal serum in the presence of soap only partially lysed the pneumococci. Mice injected with the completely lysed culture survived; mice injected with the partially lysed culture died of a typical pneumonia. The lytic action in the presence of soap was inhibited by addition of 50 per cent normal serum, but the addition of boric acid prevented the inhibitory action of serum. The mechanism advocated by Lamar was that the soap attacked the outer lipid covering of the bacterial cell making it more permeable to the antiserum.

[5] J. W. McBain, J.C.S. **121**, 621 (1922).
[6] W. D. Harkins and H. Zollman, J.A.C.S. **48**, 69 (1926).
[7] R. V. Lamar, J. Exptl. Med. **13**, 1 (1911).

Lamar then turned his attention to the hemolytic properties of the fatty acids and their soaps, studying a series of acids and their soaps including crotonic acid, its potassium soap; oleic acid, its sodium and potassium soaps; erucic acid, its potassium soap; linoleic acid, its potassium soap; chaulmoogric acid, its potassium soap; and linolenic acid and its potassium soap. He also included a mixture of soaps and acids obtained from atrophied livers of animals with experimental phosphorus poisoning. Summarizing his results with this series briefly, one can state:

1. The higher acids of the oleic series showed greater hemolytic activity than crotonic acid whose action was probably due only to its acidity.

2. The soaps were more active than the acids owing to their greater solubility.

3. Among the higher acids, there was a close relation between iodine number and hemolytic strength. High iodine number meant great hemolyzing power.

4. Configuration had some effect on hemolytic strength. Chaulmoogric acid, which is an isomer of linoleic acid, was less active even than oleic.

5. No one of the above properties alone can evaluate the hemolytic power of a compound. The greatest activity was shown by potassium linolenate, which has a combination of many carbon atoms, a straight-chain structure, high iodine number, and rather high solubility.

He also ascertained that a close parallelism existed between the lytic actions of the soaps for red blood cells and virulent pneumococci. The potassium linolenates and linoleates were respectively six and four times as active as sodium oleate. Normal goat serum inhibited the lytic action of the soaps, but the inhibition was less for those with high iodine numbers especially for the potassium soaps of the acids from liver and for potassium linolenate and linoleate.

At present, the conflict in the field of the germicidal properties of soaps is considerable. Some workers maintain that they are excellent general germicides and advocate their use as surgical antiseptics and for wound dressings, either as salves or as wet dressings. Others deny that they possess any germicidal activity whatsoever. Somewhere between these two extremes of opinion lies the happy medium. Soaps are not complete or general germicides; they are very selective in their action, that is, they kill some organisms easily and do not injure others. However, in the face of the evidence presented, one cannot say that they have no germicidal properties at all.

The toxicity of the soaps increases with the length of the carbon

chain. As long as the molecular size does not prevent the entrance of the soap into the cell, germicidal action increases with molecular weight until, eventually, the soap molecule becomes so large that it can no longer get into the bacterial cell. This causes a decrease in germicidal properties.[8]

In 1919, Larson[9] and his associates were studying the growing of bacteria in media of low surface tension. Sodium ricinoleate was found to be a convenient surface-tension depressant. Bacteria which ordinarily grew on the surface as a pellicle failed to do so if the surface tension was below 45 dynes per cm. The tendency of *Bacillus subtilis* to form spores was greatly reduced by growing on broth with a low surface tension. Thus, they conceived the idea that growing pathogens on media of low surface tension might be a method to attenuate their virulence. Pneumococci and streptococci would not grow at all in such media, but the intestinal organisms would.

This led to the actual discovery of the detoxifying properties of ricinoleate. The soap was tried on tetanus toxin, diphtheria toxin, botulinus toxin, and a culture of *Actinomyces gypsoides* which forms an endotoxin. Diphtheria and tetanus toxins were completely detoxified by enough castor oil soap (ricinoleate) to lower the surface tension to 40 dynes per cm. Guinea pigs given 100 m.l.d. when treated in this fashion developed no symptoms. *Actinomyces gypsoides* lost its pathogenicity after being mixed with 2 per cent of the soap, which destroyed its endotoxin. Botulinus toxin was resistant to the soap treatment. Larson suggested that these facts might explain why botulinus toxin is toxic by mouth whereas diphtheria and tetanus toxins are not. The latter, he said, are probably detoxified by the soaps present in the intestine and are absorbed in a harmless form. Botulinus toxin is not affected by the intestinal soaps and remains free to be absorbed in its original poisonous form.

Larson made detoxifying tests on the following sodium soaps: oleate, stearate, palmitate, myristate, and laurate, by mixing equal volumes of his standardized toxins and 1 per cent soap solutions (except where such a concentration formed a gel) and injecting the mixture into guinea pigs. Ricinoleate possessed detoxifying powers far superior to the others. The myristate and laurate detoxified somewhat, but the oleate, stearate, and palmitate offered no protection at all. In general, the detoxifying soaps were those which formed a clear or nearly clear solution in physiological saline and dialyzed readily through hardened collodion sacs. They were the better surface-tension depressants! Detoxifying power

[8] A. H. Eggerth, J. Exptl. Med. **53**, 27 (1931).
[9] W. P. Larson, J. Immunol. **25**, 41 (1919).

was proportional to solubility, and detoxification was inhibited by the presence of colloidal calcium soaps, oleates, stearates, starch, serum, or broth. In these cases, the soap was thought to be adsorbed on the foreign colloids and did not remain free to act on the toxins.

Detoxification is evidently an adsorption phenomenon, the soap being adsorbed on the toxin, causing inactivation. The toxin is merely held and not destroyed. Hence, detoxified toxins are good antigens, the toxin being slowly released over a period of time.

Larson, Halvorson, Evans, and Green[10] published a comprehensive report of their work of detoxification with ricinoleate. They find that a toxic mixture may be made non-toxic: (1) by diluting the toxin, keeping the soap concentration constant; (2) by increasing the soap concentration, keeping the toxin constant; (3) by diluting with saline to bring it to the proper soap/toxin ratio. Diluting too much causes a return to toxicity.

Aging experiments show that equilibrium between soap and toxin is reached in a short time, about fifteen minutes. The equilibrium is reversible and is disturbed by adding a salt solution. Experiments illustrated this very clearly. A soap-toxin mixture was allowed to come to equilibrium (twenty-four hours' standing). Part of it was injected into a guinea pig with no bad results. To the other part a small amount of $CaCl_2$ solution was added and the mixture injected. The pig died, indicating that the toxin had been liberated by the addition of calcium ions which precipitated the soap.

Perhaps the greatest applied field for ricinoleate, at present, is in vaccines. Larson has reported a very successful scarlet fever vaccine. He also has reported favorable results in diphtheria immunization by injecting a soap toxin mixture. The advantages claimed for this method of vaccination are that larger doses may be administered at one time, thus cutting down the number of injections necessary for immunization, and also that it eliminates the necessity of injecting any foreign serum, thus preventing serum shock and serum sickness.

At present, indications are that some of the soaps may be excellent germicides and detoxifiers. Much new work remains to be done, and much of the old work should be repeated under carefully controlled conditions before the true germicidal and detoxifying value of the soaps can be ascertained.

[10] W. P. Larson, H. O. Halvorson, R. D. Evans, and R. G. Green, Coll. Sym. Mono. **3**, 152 (1925), Chemical Catalog Co., New York.

CHAPTER III

ALCOHOLS, WAXES AND HYDROCARBONS

Alcohols. Alcohols belonging to the saturated series occur in waxes and in some fats. They are solid (higher members), white, crystallizable substances, melting without decomposition. They are not acted upon by dilute alkali or acid; on boiling with alcoholic potash and diluting the solution with water, they are precipitated unchanged. Hence they are termed "unsaponifiable."

The alcohols listed in Table VIII have been reported to have natural occurrence.

TABLE VIII

NATURAL ALCOHOLS

Name	Formula	Occurrence
Lananol	$C_{12}H_{24}O$	Wool fat
Cetanol	$C_{16}H_{34}O$	Sperm oil
Octodecanol	$C_{18}H_{38}O$	Sperm oil, rump gland wax
Arachanol	$C_{20}H_{42}O$	Demoid cysts
Carnaubanol	$C_{24}H_{50}O$	Wool fat
Hexacosanol	$C_{26}H_{54}O$	Cocksfoot
Octacosanol	$C_{28}H_{58}O$	Wheat oil
Triaccontanol	$C_{30}H_{60}O$	Lucerne
Oleanol	$C_{18}H_{36}O$	Fish-liver oils

Chibnall[1] *et al.* analyzed a series of plant and insect waxes and came to the conclusion that naturally occurring alcohols are mixtures of even-numbered alcohols from C_{24} to C_{36} and that only occasionally, in certain leaf waxes, is one particular alcohol present to the virtual exclusion of all others. They make the suggestion that names which have been given to alcohols are meaningless and should be abandoned, since these substances are mixtures of two or more alcohols.

The alcohols exhibit certain typical organic reactions. When heated with soda lime they are converted, with the evolution of hydrogen, into

[1] A. C. Chibnall, S. H. Piper, A. Pollard, E. F. Williams, and P. N. Sahai, Biochem. *J.* **28**, 2189 (1934).

the salt of the corresponding fatty acid. This reaction is used to identify the alcohols. They form esters with the fatty acids, and the resulting compound, if the alcohol is a higher one, is a wax. The term wax, however, is often used to include the mixture found in nature which is made up of true waxes, alcohols, paraffins, and higher fatty acids. The alcohols can be oxidized with chromic acid in acetic acid to the corresponding fatty acid.

Piper, Chibnall, and Williams[2] have conducted a study of the melting points and of crystal spacings, as determined by means of X-ray, of pure alcohols as well as synthetic mixtures with the idea of using this information to analyze naturally occurring alcohols. Even- and odd-numbered alcohols crystallize in an A form in which the molecules are vertical. The even-numbered alcohols crystallize also in a B form with the chains tilted about 60° to 63°. The long spacings of both the odd and even series when in the A form lie on a single straight line when plotted against the carbon number. The even-numbered alcohols crystallize from an organic solvent in the B form and in most cases adopt the A form at higher temperatures. Fig. 17 shows their data.

Wilson and Ott[3] studied the X-ray spacings of the normal alcohols from C_{10} to C_{18} at $-50°$ C. They found, as did Piper *et al.*, for the higher alcohols that there is a common, A form which both the even and odd alcohols show. They also found, however, that the even members had another form which showed a tilt of 51° 55′, and which would correspond to the C form of the fatty acids. No B form with a tilt of 60° to 63° is reported.

Piper *et al.* found the melting points of the odd and even alcohols to lie on one smooth curve, a feature characteristic of substances melting from the A form. The melting points of the synthetic mixtures of alcohols were found to be quite sharp. Fig. 18 shows their results.

The lower alcohols (from C_1 to C_8) show alternations in their melting points, which indicates that the crystal structures of the lower members are not entirely analogous to those of the higher members.

The solubility of the higher alcohols in organic solvents decreases rather strongly with increasing length of the paraffin chain. The alcohol with 26 carbons is soluble at room temperature only in chloroform and benzene but is soluble in most organic solvents at boiling, whereas the C_{36} alcohol is insoluble in all cold solvents. The alcohols are less soluble than the corresponding fatty acids.

In recent years, the relation between the plant pigments and vitamin A and related compounds has been intensively investigated by a large

[2] S. H. Piper, A. C. Chibnall, and E. F. Williams, Biochem. J. **28**, 2175 (1934).
[3] D. A. Wilson and E. Ott, J. Chem. Phys. **2**, 231 (1934).

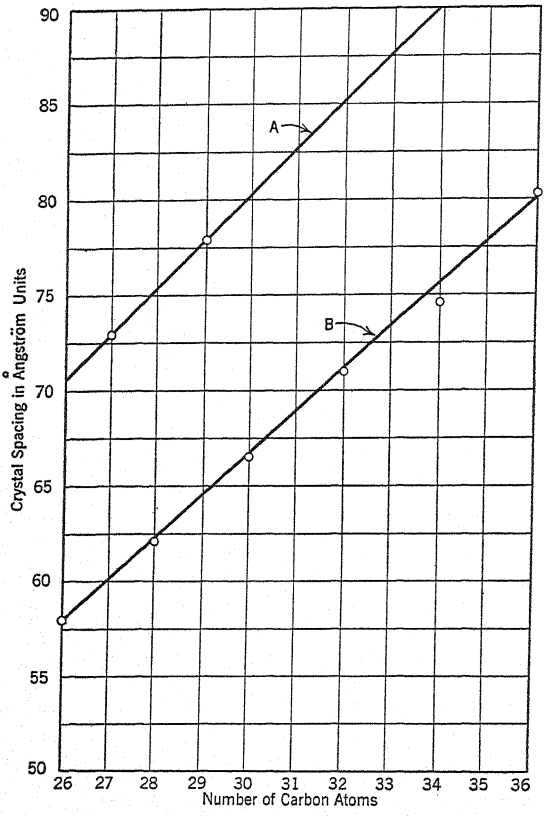


FIG. 17.—Crystal spacings of alcohols as shown by x-ray

number of workers. They have been led, however, by the researches of Kuhn in Germany, Karrer in Switzerland, and Zechmeister in Hungary.

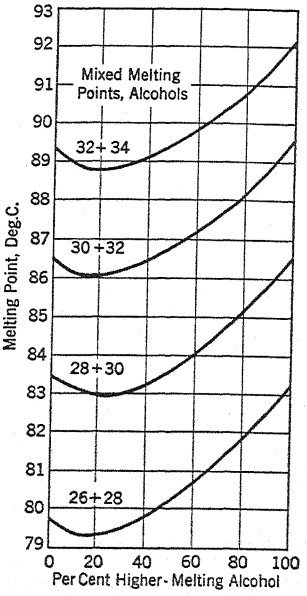


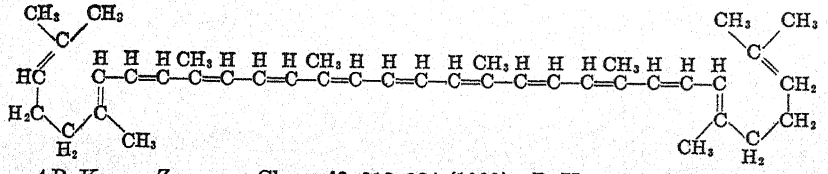
FIG. 18.—Melting points of synthetic mixtures of alcohols

Phytol, which is the alcohol in chlorophyll and occupies the same relation to chlorophyll as the globin does to hemoglobin, has the following structure:

$$CH_3CHCH_2CH_2CH_2CHCH_2CH_2CH_2CHCH_2CH_2CH_2C{=}CHCH_2OH$$
$$\quad\;\;|\qquad\qquad\;\;|\qquad\qquad\;\;|\qquad\qquad\;\;|$$
$$\quad CH_3\qquad\quad CH_3\qquad\quad CH_3\qquad\quad CH_3$$

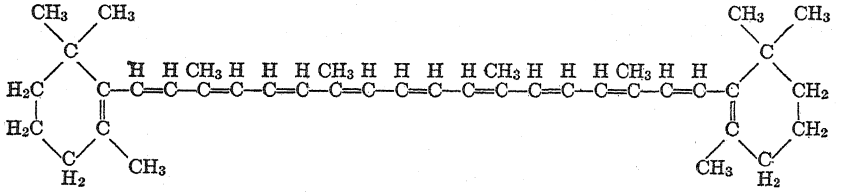
Squalene is a hydrocarbon of similar structure.

Lycopin,[4] the red pigment of tomatoes, is an unsaturated hydrocarbon having the structure:



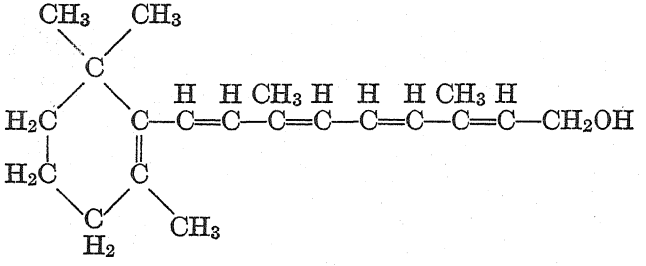
[4] P. Karrer, Z. angew. Chem. **42**, 918–924 (1929); P. Karrer and A. Helfenstein, Ann. Rev. Biochem. I, 554 (1932).

If the ring is closed and the terminal double bonds are destroyed β-carotene results.[5]

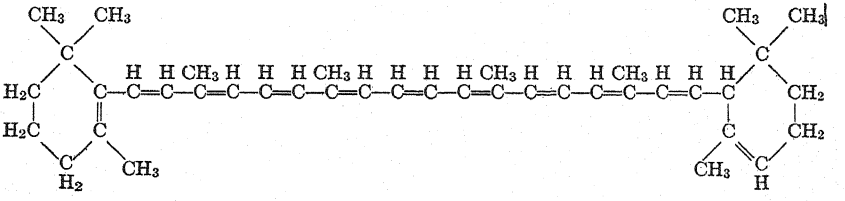


β-carotene has eleven double bonds and melts at 182°.

If we break β-carotene at the middle double bond and add the elements of water, thus making an alcohol, we obtain vitamin A:



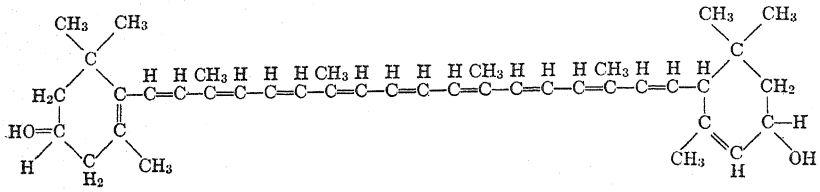
There are a number of other carotenes, among them α-carotene which has the formula:



This should be more properly called αβ-carotene.[6]

Another class of pigments, the xanthophylls, are intimately related to the carotenes. Lutein,[7] the leaf xanthophyll, has the following structure:

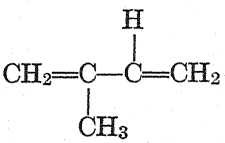
[5] P. Karrer and A. Helfenstein, Ann. Rev. Biochem. I, 554 (1932).
[6] P. Karrer *et al.*, Helv. Chim. Acta **16**, 975 (1933).
[7] P. Karrer *et al.*, Helv. Chim. Acta **16**, 977 (1933).



The xanthophylls form natural esters with the fatty acids and thereby become soluble in fat solvents. Xanthophylls have the solubility of alcohols.

Many xanthophylls and carotenes are possible, and many have been isolated. All are highly colored. The color seems to depend upon the two ring structures. One alone will not give color (for example, vitamin A).

The student should consult the recent monograph by Zechmeister [8] on the carotenoids for a thorough discussion of the relationships in this series of compounds. It is possible that they all might have arisen originally from isoprene, the hydrocarbon of rubber. It has the formula



For example, four molecules of isoprene may have condensed with one molecule of water followed by reduction to form phytol.

A study has recently been made of the X-ray diagrams of the carotenes.[9]

Research is very active in this field at the present time, and we can confidently expect many additional fundamental data on the carotinoids to appear in the near future.

The most commonly occurring alcohol is the trihydroxy alcohol, glycerol $CH_2OHCHOHCH_2OH$. It is a colorless hydroscopic thick liquid with a slight but characteristic odor and a sweet taste. Although soluble in all proportions in alcohol and water, it has very little solubility in the fats and oils and is practically insoluble in benzene, chloroform, anhydrous ether, and petroleum ether. Glycerol itself is a good solvent for many salts and other substances and dissolves many substances better than does alcohol or water. Glycerol dissolves caustic

[8] L. Zechmeister, Carotinoide, Berlin, J. Springer, 1934.
[9] G. MacKinney, J. A. C. S. **56**, 488 (1934).

alkalis, alkaline earths, and lead oxide to form chemical compounds with them. It occurs chiefly esterified with fatty acids. These esters are known as glycerides. Fats which have undergone natural hydrolysis rarely contain any detectable quantities of glycerol. It is apparent that glycerol is rapidly converted into other substances and, with very few exceptions, directly after the hydrolysis of the glycerides. Under some conditions, partial hydrolysis takes place and diglycerides are formed.

The polymer diglycerol, known as D glycerol, is prepared and used in the manufacture of low-freezing dynamite. It is made by heating glycerol with 0.2 per cent $NaHCO_3$ to about 260° C. under slightly reduced pressure for several hours. Glycerol has many industrial uses.

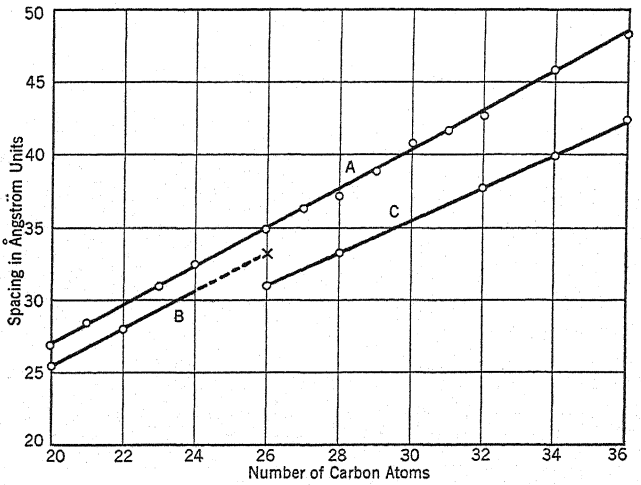


FIG. 19.—Crystal spacings of paraffins as shown by x-ray.

The Hydrocarbons. Piper *et al.*[10] have reported an extensive investigation of the synthetic and natural paraffins. The paraffins were prepared by reducing the corresponding ketone in an alcohol-water mixture with zinc and hydrochloric acid. The ketone had been obtained by heating the fatty acid in the presence of iron. Thus, myristic acid was found to give a 75 per cent yield of myristone. Other paraffins were prepared by the method of Gascard,[11] which consists of converting the proper alcohol to the iodide and then boiling the iodide in xylene with sodium and separating out the paraffin.

The paraffins were found to give the X-ray spacings shown in Fig. 19.

[10] S. H. Piper, A. C. Chibnall, S. J. Hopkins, H. Pollard, J. A. B. Smith, and E. F. Williams, Biochem. J. **25**, 2072 (1931).

[11] A. Gascard, Ann. Chim. (9) **15**, 332 (1921).

The paraffins show three long crystal spacings, A, B, and C. Unlike those of the alcohols and fatty acids, the spacings correspond to only one molecular length. The shorter hydrocarbons up to, and including, hexacosane, show the A and B spacings which indicate tilts of 90° and 63°, respectively. Hexacosane also exhibits the C form with a tilt of 53°. All hydrocarbons higher than hexacosane show only the A and C crystal modifications. The odd-carbon-atom paraffins, however, never show the C form, only the A.

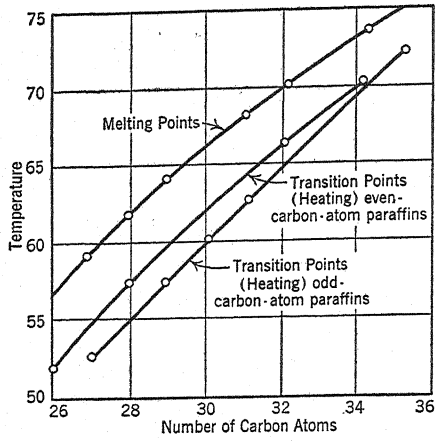


FIG. 20.—Melting points and transition points of paraffins.

The paraffins have transition points as well as melting points. The transition point is especially characteristic and, being very sensitive to impurities, is useful in the analysis of the paraffins. The melting points of the paraffins from C_{26} to C_{32} lie on a smooth curve. Apparently there

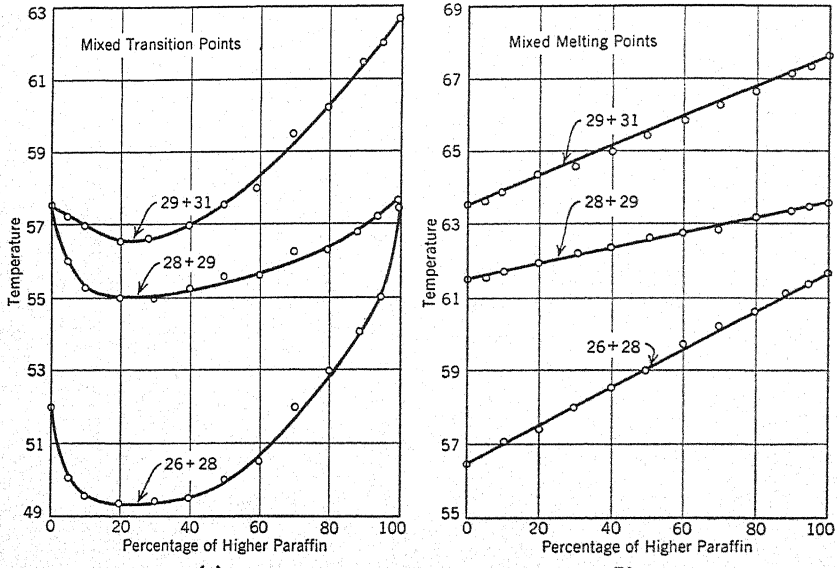


FIG. 21.—Transition points and melting points of mixed paraffins.

are two transition points, but the second one is difficult to observe and is not affected by impurities. Fig. 20 shows the melting point and the first transition temperatures of the paraffins as obtained by Piper *et al.*

The transition of the odd-carbon-atom paraffins presumably involves a change of cross section of the cell without affecting the molecular tilt. This explains why the X-ray does not show two crystal forms of the odd-carbon-atom hydrocarbons but only the A form.

The melting point and especially the transition points are useful in the analysis of an unknown paraffin. The melting point of a mixture of two paraffins lies along a straight line, *i.e.*, the melting point is a function of the mean molecular weight, while the transition points are often lower than either of the two components. Fig. 21 shows the results Piper *et al.* obtained with mixed transition and mixed melting points. For binary mixtures of neighboring homologues, both the melting points and the transition points are as sharp as those for pure specimens. If, however, the paraffins have widely different molecular weights, or if the mixture is made up of three or more individuals, this is not true. With mixtures, the transition point occurs at a lower temperature than it does for a pure substance of a given melting point, *i.e.*, the melting points and transition points are farther apart with mixtures than they are for pure hydrocarbons.

Table IX shows the names, melting points, and number of carbon atoms in some of the naturally occurring and synthetic paraffins.

TABLE IX

Name	Melting Point	Number of Carbon Atoms
n-Hexacosane	56.4	26
n-Hentriacontane	59	27
n-Octacosane	61.4	28
n-Nonacosane	62.7	29
n-Triacontane	65.6	30
n-Hentriacontane	67.6	31
n-Dotriacontane	69.5	32
n-Tetratriacontane	72.5	34
n-Pentatriacontane	74.4	35
n-Hexatriacontane	75.7	36

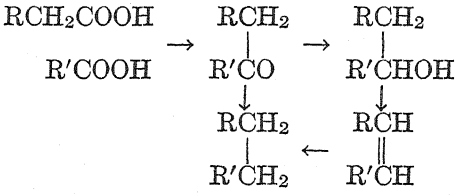
The fatty acid, paraffin, and alcohols contained in waxes are shown in Table X. [14]

TABLE X

Constituents of Various Plant and Insect Waxes

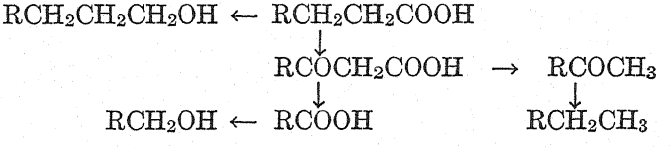
	n-Fatty Acids						Primary Alcohols							Paraffins							*n*-Nonacosan-10-ol	*n*-Nonacosan-15-ol	*n*-Nonacosan-15-one	Cocceryl Alcohol	13-Keto-*n*-dotriacontanoic Acid
	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}	C_{34}	C_{24}	C_{26}	C_{28}	C_{30}	C_{32}	C_{34}	C_{36}	C_{25}	C_{27}	C_{29}	C_{31}	C_{33}	C_{35}	C_{37}					
Insect waxes:																								
Lac wax (*Coccus lacca*)	...	...	...	+	+	+	...	+	+	+	+	+	+												
Cochineal wax (*Coccus cacti*)	...	...	...	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	+
Chinese insect wax (*Coccus cerefera*)	...	+	+	+	...	...	...	+	+	+															
Beeswax (*Apis mellifera*)	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+								
Leaf cuticle waxes:																									
Carnauba wax	...	+	+	+	+	+	...	+	+	+	+	+		...	+										
Candelilla wax	...	...	+	+	+	+	...	...	+	+	+	+	...	...	...	...	+	+							
Raphia wax	...	...	...	...	...	...	...	...	+	+	+			...	...	...	...	...	...	...					
Pisang wax	...	...	...	...	...	...	...	...	+	+	+			...	...	...	...	...	...	...					
Fruit and seed coat cuticle waxes:																									
Apple cuticle wax	...	+	+	+	+	...	...	+	+	+	...	...	...	...	+	+	...	...	...	...	+				
Cotton wax	+	+	+	+	+	+	+	+	+	+	+	+	...	...	...	...	?								
Leaf waxes (non-cuticular):																									
Brussels sprout	?	+	+	?	...	...	...	+	+	+	+	...	...	...	...	+	+	...	...	...	...	+	+		
Cabbage	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	+	...	...	...	...	?	+		
Swede turnip	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+	+	...	...	...	...	+			
White mustard	...	+	+	+	...	...	...	...	+	+	+	...	...	...	...	+	+	...	...	...	...				
Cactus	...	...	?	?	?	...	...	+	+	+	+	...	...	...	...	...	...	+	+	+					
Tobacco	...	...	...	...	...	...	...	...	...	...	...	...	...	?	?	+	+	+							
Wild white clover	...	+	+	+	...	...	...	...	+	+															
Spinach	+	+	...	...	...	...	...	...	...	...	...	...	...	...	...	...	+								

Channon and Chibnall[12] were the first to suggest that the paraffins arise from a reduction of the corresponding ketone which in turn was produced from two molecules of fatty acid, according to the following scheme which would also account for the presence of the alcohol:



Collison and Smedley-Maclean[13] support this hypothesis and present additional evidence. They point out that only paraffins with odd numbers of carbon atoms have been found in nature. Hentricontane ($C_{31}H_{64}$) has been found very frequently and, presumably, is derived from palmitic acid by conversion of the latter to palmitone which is then reduced to the paraffin. The ketones have never been identified as constituents of waxes, however.

Chibnall and Piper[14] have reconsidered the question and were able to show that paraffins are always accompanied by fatty acids of chains of approximately the same length. They were, therefore, led to suggest the following set of reactions:



The serious difficulty with this scheme is that the methyl ketone has never been found to have natural occurrence. Chibnall and Piper further believe that the hydroxy and ketonic acids may be derived from the unsaturated higher acids and also that this is why the unsaturated higher acids are missing in the waxes. By these reactions, the components of all the numerous waxes can be readily derived from a few members of a series of unsaturated acids having an even number of carbon atoms.

The plant and insect waxes fall into three main classes: (1) insect secretions, (2) plant cuticle secretions, and (3) substances which are a part of the general fat phase of the cell.

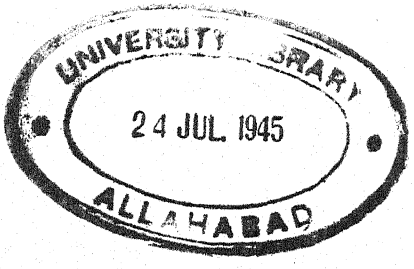
[12] H. J. Channon and A. C. Chibnall, Biochem. J. **23**, 168 (1929).
[13] D. L. Collison and I. Smedley-Maclean, Biochem. J. **25**, 606 (1931).
[14] A. C. Chibnall and S. H. Piper, Biochem. J. **28**, 2208 (1934).

The liver oils contain appreciable quantities of paraffin, and these are often highly unsaturated. Channon *et al.*[15] have reported on a study of the pig-liver hydrocarbons. Squalene was not found. The presence of considerable hydrocarbons of great molecular weight and of a high degree of unsaturation is reported.

Chibnall *et al.*[16] studied the wax constituents of the apple cuticle. *n*-Nonacosane, *n*-heptacosane, *d*-10-nonacosanol, *n*-hexacosanol, *n*-octacosanol, and *n*-triacontanol were found.

Pollard *et al.*[17] analyzed the waxes of grasses. They found the unusual case of a practically pure alcohol in cocksfoot which turned out to be *n*-hexacosanol.

[15] H. J. Channon, J. Devine, and J. V. Loach, Biochem. J. **28**, 2012 (1934).
[16] A. C. Chibnall, S. H. Piper, A. Pollard, J. A. B. Smith, and E. F. Williams, Biochem. J. **25**, 2095 (1931).
[17] A. Pollard, A. C. Chibnall, and S. H. Piper, Biochem. J. **25**, 2111 (1931).

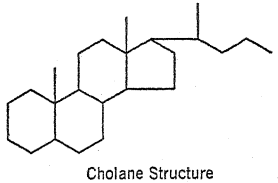


CHAPTER IV

THE STEROLS AND RELATED COMPOUNDS

Recently, excellent reviews of the chemistry and physiology of the sterols and related compounds[1] have appeared; all that will be attempted in this chapter is to outline the relation between the sterols, bile acids, sex hormones, and calciferol.

The word sterol, derived from the Greek, means solid alcohol. Sterols are found both in plants and in animals. The ring structure of the sterols is one of the most massive with which living things have to deal. The so-called cholane structure is parent of this series of compounds.



Cholane Structure

Table XI gives a list of the sterols which have been identified.

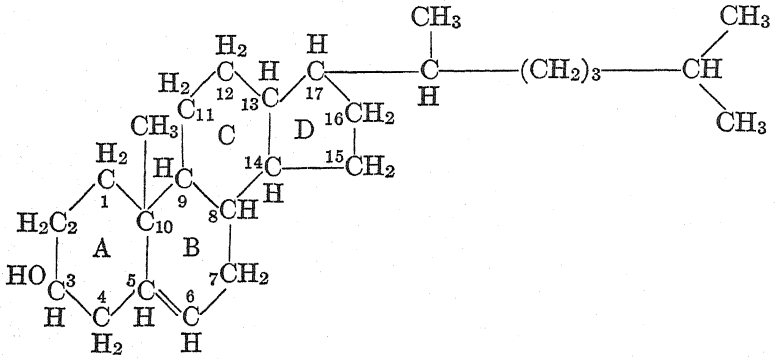
TABLE XI

Name	Formula	Double Bonds	Occurrence
Cholesterol	$C_{27}H_{46}O$	1	All animal cells
Dihydrocholesterol	$C_{27}H_{48}O$	0	Accompanies cholesterol
Coprostanol	$C_{27}H_{48}O$	0	Feces
Ostreasterol	$C_{29}H_{48}O$	2	Oysters, gastropods
Lanosterol	$C_{30}H_{50}O$	2	Wool fat
Agnosterol	$C_{30}H_{48}O$	3	Wool fat
Ergosterol	$C_{28}H_{44}O$	3	Ergot, yeast
γ-Sitosterol	$C_{29}H_{50}O$	1	Fats of higher plants
Stigmasterol	$C_{29}H_{48}O$	2	Calabar bean, soy bean
Cinchol	$C_{29}H_{50}O$	1	Cinchona bark
Fucosterol	$C_{29}H_{48}O$	2	Algae
Zymosterol	$C_{27}H_{44}O$	2	Yeast

[1] L. F. Fieser, The Chemistry of Natural Products Related to Phenanthrene, A.C.S. Monograph, Reinhold Publishing Corporation, New York 1936; Harry Sabotka, Chem. Rev. **15**, 311 (1934); C. E. Bills, Physiol. Rev. **15**, 1 (1935).

Cholesterol. The chief animal sterol is cholesterol, which is apparently a constituent of all animal cells and is abundant in nervous tissue. We shall consider cholesterol as a typical sterol and treat it in some detail. Windaus[2] early proposed a structure having three six-membered rings and one five-membered ring. The X-ray studies of Rosenheim and King[3] necessitated a revision of the structure. Although the structure as proposed by these workers was not adopted, Wieland and Dane,[4] on the basis of these studies and of the organic reactions, suggested a structure for dihydrocholesterol.

The structure finally arrived at for cholesterol is



Isomerism. At least two kinds of steroisomerism are encountered in sterol chemistry. We have isomerism between the cholestane and coprostane series. Cholestane, which is the saturated hydrocarbon corresponding to cholesterol, has the hydrogen on carbon 5 in a *trans* relation to the methyl on carbon 10; coprostane has these groups *cis* to each other. The *trans* series are sometimes prefixed by *allo-*.

The other point at which steroisomerism arises is a *cis: trans* relation between the hydroxyl group on carbon 3 and the hydrogen on carbon 5. All natural sterols are of one kind and are by convention called the *trans* isomer. They are spoken of as of the β-type. The *cis* isomers are called the *epi* type.

As indicated above, the differences between the cholestane and coprostane series arises in rings *A* and *B*. The old system of nomenclature was not based on structural relations. Rosenheim and King[5] have proposed a new system of nomenclature based on such relations:

[2] A. Windaus, Abderhaldens Handb. biol. Arbeitsmeth., Abt. 1, Teil 6, Heft I, 169 (1922).
[3] O. Rosenheim and H. King, J. Soc. Chem. Ind. **51**, 954 (1932).
[4] H. Wieland and E. Dane, Z. physiol. Chem. **210**, 274 (1932).
[5] O. Rosenheim and H. King, Ann. Rev. Biochem. **III**, 90 (1934).

Proposed Nomenclature	Old Nomenclature
Coprostanol (saturated alcohol)	Coprosterol
Coprostenol (unsaturated alcohol)	Allocholesterol
Coprostanone (saturated ketone)	Coprostanone
Coprostenone (unsaturated ketone)	Cholestenone
Coprostane (saturated hydrocarbon)	Pseudo cholestane
Coprostene (unsaturated hydrocarbon)	Psuedo cholestene

The so-called isocholesterol of lanolin is a mixture of agnosterol and lanosterol, neither of which is isomeric with cholesterol.

Preparation. Cholesterol can be prepared from brain tissue by mixing the tissue with anhydrous calcium sulfate, powdering the hard mass, extracting it with ether, and purifying the crude cholesterol. A better source for cholesterol is gallstones. Some gallstones are calcium carbonate-bile pigment, but others are almost pure cholesterol.[6] Cholesterol, as it occurs, generally contains large quantities of cholesterol ester, and this must be hydrolyzed. Anderson's method is recommended.[7]

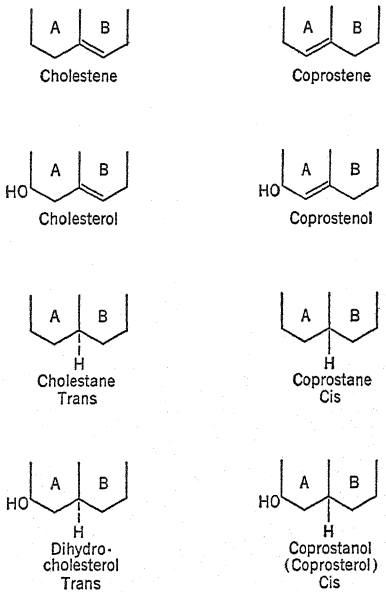


FIG. 22.—Ring structure of the cholestane and coprostane series.

Color Reactions. Cholesterol yields, as is general with the sterols, several color reactions. The Liebermann-Burchard reaction is given when cholesterol is treated with chloroform, acetic anhydride, and sulfuric acid; it consists of a violet color which varies in intensity with the concentration of cholesterol and is the basis for a quantitative method.[8] Most sterols, however, give the same, or very nearly the same, color as cholesterol, which limits the usefulness of the reaction. Other color reactions are Salkowski's reaction (purple color with chloroform and sulfuric acid), Schiff's reaction (xantho reaction with nitric acid and ammonia), Lifchütz's reaction (oxidation with benzoyl peroxide to oxycholesterol then color changes with sulfuric acid).

[6] J. E. Sweet, Colloid Symposium Ann. VII, 248 (1930); H. B. Weiser and G. R. Gray, Arch. Path. **17**, 1 (1934).

[7] R. J. Anderson, J.B.C. **71**, 407 (1927).

[8] W. R. Bloor, J.B.C. **29**, 437 (1917).

Digitonide. Cholesterol can also be estimated gravimetrically with digitonin, with which it forms a precipitate insoluble in alcohol. It is necessary to precede the precipitation of the digitonide by saponification. Care must be exercised since cholesterol is changed by prolonged treatment with alkali so that it cannot be precipitated by digitonin. The saponification should be carried out in the absence of air. The only substances in plant or animal tissue which are precipitable by digitonin are cholesterol, stigmasterol, sitosterol, and possibly other phytosterols and coprostanol.

It is possible, instead of weighing the precipitated digitonide, to oxidize it with a dichromate-sulfuric acid mixture and thus transform the gravimetric into a volumetric method.[9]

Schoenheimer and Sperry[10] have combined the digitonin precipitation with a modified Liebermann-Burchard reaction and found the method to be accurate and rapid. The virtue of the digitonin precipitation is that chromogenic substances other than cholesterol are removed.

Recently, Sobel, Drekter, and Natelson[11] have developed a quantitative method for cholesterol based on the insolubility of pyridine cholesteryl sulfate in petroleum ether. The precipitation is carried out in benzene, and only that cholesterol with completely free OH reacts. The precipitated cholesterol is then estimated by the Liebermann-Burchard reaction.

Physical Properties. An important property of cholesterol is its ability to confer on a fat or oil the capacity to absorb relatively large quantities of water. The sterol is not hydrophilic but actually hydrophobic. The water-absorbing ability is, no doubt, due to its tendency to form water-in-oil emulsions. In this connection, it is interesting to consider so-called metacholesterol. Its exact status is a doubtful one although there have been numerous references to it.[12]

Apparently the substance can be isolated in fairly large amounts from preparations of ordinary cholesterol by virtue of its colloidal solubility in aqueous solutions of alcohol. It crystallizes in elliptical platelets and melts at 139° to 141° (ordinary cholesterol melts at 149°). It is claimed to be hydrophilic, but the sole justification for this statement seems to be that it forms colloidal suspensions in water, which, of course, is no reason at all. One cannot but suspect that it is a crystal modification of ordinary cholesterol. The substance is said not to occur in the

[9] R. Okey, J.B.C. **88**, 367 (1930).
[10] R. Schoenheimer and W. M. Sperry, J.B.C. **106**, 745 (1934).
[11] A. E. Sobel, I. J. Drekter, and S. Natelson, J.B.C. **115**, 381 (1936).
[12] I. Lifschütz, Biochem. Z. **280**, 65 (1935); Arch. Pharm. **265**, 450 (1927); Z. physiol. Chem. **114**, 108 (1921).

oil of eggs and, supposedly, is mixed about half and half with cholesterol in the brain. All the blood sterol was reported to be metacholesterol.

Actually, it is comparatively easy to prepare colloidal suspensions of ordinary cholesterol, and Moyer[13] has reported in detail on this problem. He determined the electrophoretic speeds of the cholesterol particles in acetate buffer and found them to vary considerably, according to the method of preparation. Later, he added ergosterol[14] to his study and found, rather surprisingly, that the ergosterol had exactly the same electrophoretic mobility as cholesterol. He prepared his sterol suspensions by grinding the sterol with ice at $-10°$ C. and suspending this material in the proper buffer. His results are shown in Fig. 23.

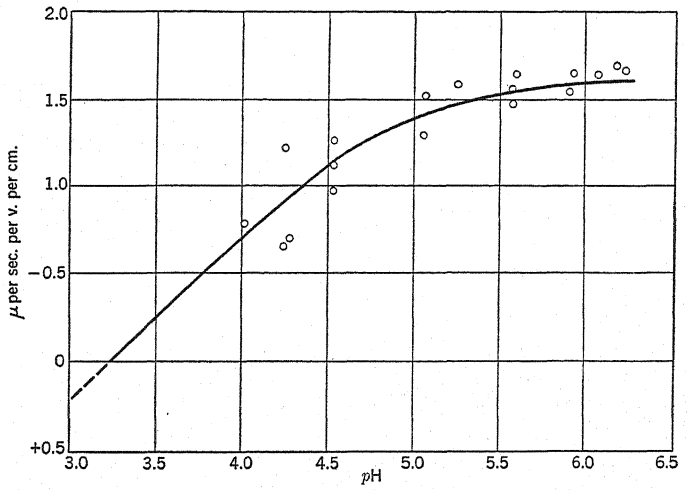


FIG. 23.—Electrophoretic mobility of ergosterol and cholesterol. The circles are the experimental points for ergosterol; the smooth curve is the theoretical curve for cholesterol.

As shown in Fig. 23, cholesterol has a definite isoelectric point. Perhaps this is due to the presence of the double bond which tends to attract hydrogen ions and renders the cholesterol positive in acid solutions.

Remesow and Sepalowa[15] report that cholesterol sols have strong reducing properties, a 5 per cent sol corresponding to about a 63 mg. per cent and a 10 per cent sol to a 108 mg. per cent pure glucose solution.

Physiology. There seems to be ample evidence that the animal body is capable of synthesizing cholesterol. Sitosterol and other plant

[13] L. S. Moyer, Biochem. Z. **273**, 122 (1934).
[14] L. S. Moyer, J. Gen. Physiol. **18**, 749 (1935).
[15] I. Remesow and O. Sepalowa, J. Biochem. (Japan) **22**, 71 (1935).

sterols are not absorbed by animals even when fed along with bile acids. Allocholesterol is absorbed somewhat less readily than cholesterol. Cholesterol when fed alone is absorbed very little, if at all, but when mixed with fat is fairly well absorbed. Saturation of the double bond prevents absorption. Coprostanol is not absorbed. Ergosterol is not absorbed, but its irradiated product, calciferol, is absorbed. In view of the fact that the plant sterols are not absorbed, they cannot be regarded as the mother substance of cholesterol. The suggestion has been made that it is formed from squalene or from carotene.[16]

Toxic effects are produced in herbivora by cholesterol feeding, a condition known as atherosis resulting, which is similar to human atherosclerosis. The carnivora and omnivora are apparently able to protect themselves by destruction of excess cholesterol or by excreting it. Although the feeding of cholesterol to rats brings about an accumulation of free cholesterol and cholesterol esters in the liver, this condition can be prevented by a simultaneous feeding of choline.

No satisfactory answer can be given as to the place of origin of cholesterol; to all appearances, it is formed in the cells in which it occurs.

Sperry[17] reports a very large number of determinations of total and free cholesterol in blood serum of normal humans. He encountered considerable variation in the total sterol, but the free sterol made up a constant fraction of the total and was within extremely narrow limits 26.9 per cent. This possibly means that the hydrolysis of the sterol ester is at equilibrium in the blood. In order for this to be true, it would be necessary for the percentage of free fatty acid of the blood to be constant. Page *et al.*[18] disagree with the findings of Sperry and report a variation of free cholesterol from 22.4 to 72.3 per cent of the total sterol. The reason for this disagreement is not known.

Drekter, Sobel, and Natelson,[19] using their new method of determining cholesterol as the pyridine cholesteryl sulfate, have found only about one-third the free cholesterol usually reported for the blood serum by the digitonin precipitation. They separate cholesterol into four fractions: "unbound cholesterol," which is that cholesterol precipitated as the pyridine cholesteryl sulfate; "free cholesterol," the fraction precipitated by digitonin; "loosely bound" cholesterol, the difference between these two; and ester cholesterol, the difference between total and free cholesterol.

[16] H. J. Channon, J. Devine, and J. V. Loach, Biochem. J. **28**, 2012 (1934).
[17] W. M. Sperry, J.B.C. **114**, 125 (1936).
[18] I. H. Page, E. Kirk, W. H. Lewis, W. R. Thompson, and D. D. Van Slyke, J.B.C. **111**, 613 (1935).
[19] I. J. Drekter, A. E. Sobel, and S. Natelson, J.B.C. **115**, 391 (1936).

They find that most of the cholesterol is unbound in the red cells, whereas in normal serum a relatively small percentage of the cholesterol is unbound. Incidentally, they confirm the findings of Sperry that there is a constant ratio between the free and total cholesterol in the blood serum.

If we can generalize our somewhat meager information, we can state that the presence of free sterol seems to be associated with fat accumulation and a decrease in free sterol leads to fat metabolism and dispersion. The significance of this will be discussed in the chapter on emulsions.

There has been considerable speculation regarding the exact physiological rôle which cholesterol plays. Free cholesterol is known to act, at least *in vivo*, as a neutralizing substance to a number of different poisons such as saponin and tetanolysin, and as a hemolytic agent of the pneumococcus. It also has strong inhibitory action towards hemolytic substances like the unsaturated fatty acids. Cholesterol exerts a neutralizing and inhibiting action on the lipolytic enzymes, and thus may help to regulate the rate of lipid digestion within the cell. Mention has already been made of the capacity it gives to neutral fat to absorb and hold water. As yet, we know very little that is certain concerning the rôle of cholesterol in vital processes.

Plant Sterols. A number of sterols have been isolated from plants. They are grouped under the general name phytosterols. In this group are sitosterol ($C_{27}H_{44}O$) which is the principal plant sterol, brassicasterol ($C_{28}H_{46}O$) from rape oil, stigmasterol ($C_{30}H_{50}O$) from calabar bean fat, and ergosterol from the fat of yeast and other fungi. Cholesterol, ergosterol, sitosterol, and stigmasterol have identical ring systems differing only in the number of double bonds. Sitosterol is really a mixture of three isomers, α, β, and γ, which are difficult to separate.

MacLachlan[20] has studied the free and combined sterol as well as the total fatty acids in germinating soy bean seed. The total fat decreased considerably while there was a continuous synthesis of sterol. Table XII has been calculated from MacLachlan's data. The milligram percentage was invariably calculated on the dry weight of the original seeds. It was necessary to do this because there was appreciable loss of dry weight as the seeds germinated and the amount on a dry-weight basis of the germinated seeds would obviously not give a fair idea of the sterol changes.

The indicated increase in the total sterol in the cotyledons is within the experimental error so that we can consider the total sterol in the cotyledons to remain constant. The large increase for both the free and combined sterol comes in the roots, stems, and leaves. The striking

[20] P. L. MacLachlan, J.B.C. **113**, 197 (1936); **114**, 185 (1936).

TABLE XII

	Total Sterol in Milligram Per Cent	Free Sterol in Milligram Per Cent
Ungerminated seeds	97.0	81.5
Roots, stems, and leaves	162.5	146.0
Cotyledons	103.3	40.3

result is the sharp decrease in the free sterol in the cotyledons over that in the seeds. This is in keeping with the suggestion made above that free sterol is associated with a fat accumulation.

The cardiac aglycones are closely related to the sterols. They possess, however, a lactone ring attached to carbon 17 of the cholane structure. There are definite indications that the cardiac effect is intimately associated with the unsaturated lactone ring.

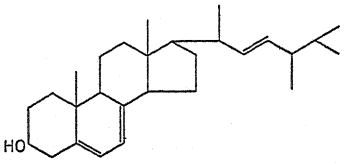
Vitamin D. Rosenheim and Webster, Steenbock and Black, and Hess, Weinstock, and Helman discovered[21] independently and almost simultaneously that more or less pure samples of cholesterol, when irradiated with ultra-violet light, acquired the property of replacing the antirachitic vitamin D in the diet of animals. It was found that many foods showed this property. It was shortly discovered that, when cholesterol had been purified by chemical methods, it largely lost this property.

It was noted that, after irradiation, not all the sterols could be precipitated by digitonin. It was also found that only those sterols which were obtained directly from plant or animal cells had the power to replace vitamin D. It was further observed that, when cholesterol had been purified by chemical means, it no longer possessed a characteristic absorption band in the ultra-violet. It appeared, therefore, as though some impurity were present in cholesterol purified by physical means and that the presence of this impurity accounted for the characteristic ultra-violet absorption band and for the antirachitic properties of cholesterol. To bring a long story to a short ending, it was found that ergosterol was this impurity, which, when irradiated, yielded calciferol.

Although the transformation of ergosterol into calciferol must be regarded as a simple unimolecular rearrangement, it is now clear that

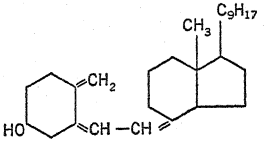
[21] O. Rosenheim and T. A. Webster, Lancet **208**, 1025 (1925); H. Steenbock and A. Black, J.B.C. **64**, 263 (1925); A. F. Hess, M. Weinstock, and F. D. Helman, J.B.C. **63**, 305 (1925).

other transformation products are formed concurrently, and it seems very probable that a number of inactive substances are produced. Calciferol has three double bonds as has ergosterol, but at least one of these bonds is in a position different from that of the ergosterol. There seems to be an opening up of the molecule. Windaus *et al.*[22] have suggested the following structure for ergosterol:



Ergosterol

The following structure has been proposed for the structure of calciferol:



Calciferol

For details of calciferol, Askew *et al.* should be consulted.[23] Briefly, the process consists of irradiating ergosterol in an organic solvent, removing the solvent, separating the unchanged ergosterol from the active resin, esterifying this with dinitrobenzoyl chloride, and hydrolyzing the dinitrobenzoate that separates in crystalline form from the mixtures of esters. In appearance, calciferol is indistinguishable from ergosterol. It is more unstable than ergosterol and after nine months' exposure becomes a brown resin. It is strongly dextrorotatory and gives no precipitate with digitonin. For details concerning the properties of ergosterol and calciferol, one should consult papers by Bacharach *et al.*[24]

When ergosterol is irradiated a number of substances are obtained; the compounds formed depend on the wave lengths of light used. The following is one sequence encountered:

[22] A. Windaus, H. H. Inhoffen, and S. V. Reichel, Liebig's Ann. **510**, 248 (1934)

[23] F. A. Askew, R. B. Bourdillon, R. K. Callow, J. S. L. Philpot, and T. A. Webster, Proc. Roy. Soc. **109B**, 488 (1932).

[24] A. L. Bacharach, E. L. Smith, and S. G. Stevenson, Analyst **58**, 128 (1933).

Ergosterol
↓
Lumisterol
↓
Tachysterol
↓
Calciferol
↓
Substance 248
↙ ↘
Suprasterol I Suprasterol II

Danielli and Adam[25] have investigated surface films of ergosterol and irradiation products of ergosterol on 0.01*N* HCl and various strengths of

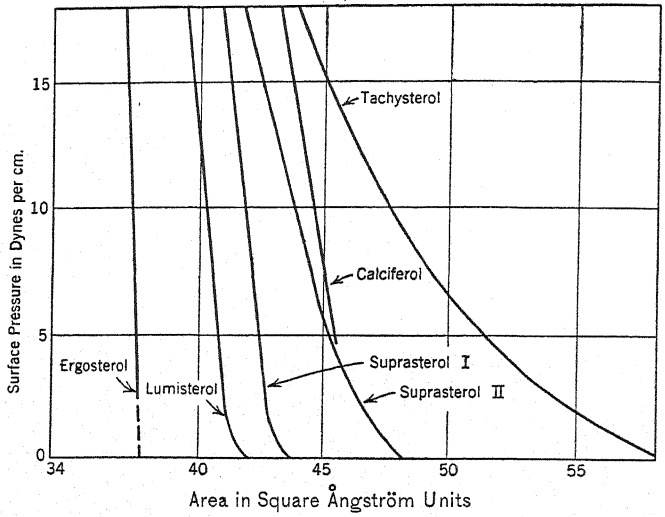


FIG. 24.—Surface pressure-area curves of ergosterol and some of its irradiation products on 0.01 *N* HCl.

potassium permanganate. The substances investigated were ergosterol, lumisterol, tachysterol, calciferol, and two "suprasterols," the last five being formed successively during irradiation of ergosterol. In addition to these, the following were investigated: pyrocalciferol, formed by the action of heat on calciferol; α-dihydroergosterol, in which one of the double bonds in the ring system of ergosterol is hydrogenated; α- and β-ergosterols, in which the double bond in the side chain is hydrogenated in addition to one of those in the ring system; α- and β-ergostdienetriols, formed by the action of perbenzoic acid on ergosterol at low and high temperatures. Fig. 24 shows the results obtained.

[25] J. F. Danielli and N. K. Adam, Biochem. J. 28, 1583 (1934).

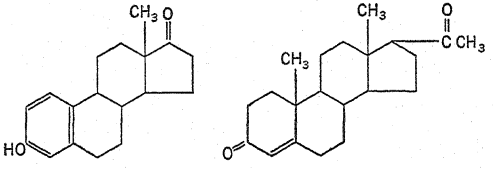
Recently, Koch, Koch, and Ragins[26] reported that pro-vitamin D is not limited to ergosterol but can be formed from cholesterol. Yoder[27] reports an antirachitic substance produced from cholesterol but different from calciferol. It was isolated in pure form.

Vitamin D from fish oils does not seem to be identical with calciferol from irradiated ergosterol. In fact, there is evidence that the ability of a sterol to be activated depends upon the presence of conjugated double bonds in the ring system, and accordingly several substances which possess antirachitic activity are to be expected.

Apparently, calciferol regulates the passage of calcium and phosphorus across the intestinal wall. The net retention of calcium and phosphorus is the resultant of two opposing factors: (1) increased absorption, (2) increased excretion by the kidneys. Vitamin D increases the diffusible fraction of calcium and the non-diffusible and diffusible phosphorus.

Sex Hormones. This phase of the problem has had almost its entire growth during the past six years, which certainly is an amazing achievement.

The female hormones which have so far been identified and whose chemical structure is known are two in number oestrone and progestrone. Oestrone is concerned with oestrus in the female animal, which involves a growth of the mucosa in the uterus. This hormone is derived from the ovary. The second female hormone, progesterone, is found in the corpus luteum and prepares the mucosa for the implantation of the fertilized ovum. The action of oestrone is not very specific as there are several substances with a similar structure which will give the same physiological reaction. Progesterone, on the other hand, is highly specific. The two hormones have the following structure:

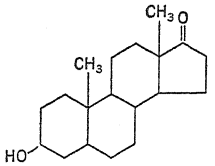


Oestrone Progestrone

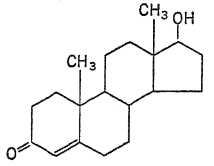
The male hormones so far identified are also two in number. Androsterone, which was isolated and identified first, is obtained from urine. Testosterone was isolated from testes and is more potent than andro-

[26] F. C. Koch, E. M. Koch, and I. K. Ragins, J.B.C. **85**, 141 (1929).
[27] L. Yoder, Sci. **80**, 385 (1934).

sterone. Both hormones are responsible for the development of the male genitalia and the secondary male sex characters. They have the following chemical structure:



Androsterone

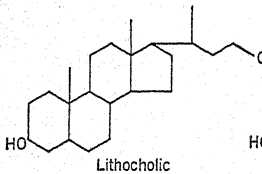


Testosterone

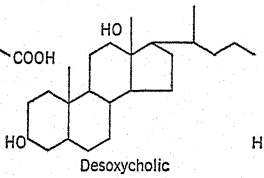
The present state of investigation leads to the conclusion that the sex hormones are oxidation products of the bile acids and sterols. The process involves the breakdown of the side chain.

It is believed that metabolic substances originating from cholesterol, bile acids, or sex hormones may be concerned directly or indirectly in the production of malignant tumors. It has been found that many substances having the phenanthrene nucleus will give rise to cancer in mice.

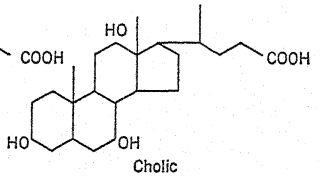
Bile Acids. Bile contains a variety of substances, among them the bile acid salts. The three most important simple bile acids, in order of increasing abundance in bile, are (1) lithocholic, (2) desoxycholic, and (3) cholic. The structural formulas of these acids are:



Lithocholic



Desoxycholic



Cholic

Cholic acid forms compound bile acids which, apparently, play a more important physiological rôle than the simple acids. Glycocholic acid is an ester of cholic acid and glycine, and taurocholic acid is a similar compound composed of cholic acid and taurine.

There is considerable speculation as to how these bile acids are formed. Certain bacteria bring about the following reactions:

Coprostanol (Coprosterol)
↓
Coprostane (Pseudo cholestan)
↓
Cholanic acid

Monohydroxycholanic acid	Dihydroxycholanic acid	Trihydroxycholanic acid
Lithocholic acid	Desoxycholic acid	Cholic acid

Schoenheimer *et al.*[28] conducted an ingenious experiment employing deuterium to "tag" molecules fed to rats. They were able to demonstrate the biological conversion of coprostanone to coprostanol. They suggest that coprostanol is formed from cholesterol by oxidation of cholesterol to the ketone, which, in turn, is converted into coprostanol.

It was formerly thought that fat was saponified to glycerine and soaps in the intestines. McClendon,[29] however, has shown that the contents of the intestines are acid, the *p*H rising in the lower levels, but even in the ileum it rarely is above neutrality. Only a fraction of the fatty acid can exist as soap at these reactions, which means that there is considerable free acid to be considered. The mechanism involved is still uncertain. Apparently the bile acids play a major rôle, especially the complex bile acids. A good review of this subject has been given by Verzar.[30]

When fatty acids are brought in contact with a large excess of taurocholic and glycocholic acids, clear solutions result which are stable not only in alkaline buffer solutions but also at *p*H values as low as 6.2. The solutions so formed are also able to diffuse through dialyzing membranes. A new significance is thus given to the rôle of bile acids in fat digestion and absorption. The earlier view was that they merely emulsified the fats by virtue of their surface-tension-lowering properties, so that the water-soluble lipase could more effectively attack the neutral fats. Actually, however, the bile acids appear to exert a much more marked effect on the fatty acids than on neutral fats. This action seems to be of the same nature as hydrotropism, a term invented by Neuberg to describe the property which certain substances have of bringing other insoluble substances into solution. The hydrotropic substances are of diverse types and are extensively used in pharmaceutical chemistry.

Apparently, after the fatty acids are rendered soluble by the bile salts, they diffuse to the intestinal mucosa where they are absorbed; the bile salts are separated from the fatty acid and returned to the intestine where they again fulfil their mission. Thus a relatively small amount of bile salt is able to render considerable fatty acid diffusible.

[28] R. Schoenheimer, D. Rittenberg, and M. Graff, J.B.C. **111**, 183 (1935).
[29] J. F. McClendon, Am. J. Physiol. **38**, 191 (1915).
[30] F. Verzar, Nutrition Abstracts and Reviews, **2**, 44 (1933).

CHAPTER V

FATS AND OILS

The fats and oils are the fatty acid esters of the trihydroxy alcohol, glycerol. If the ester is liquid at ordinary temperature, it is said to be an oil; if solid, a fat.

Pure triglycerides are colorless, odorless, and tasteless. The odor of natural fat is due to foreign substances.

The correct approach to the study of the fats and oils is by way of the synthetic products, so that we know with what we are dealing; the natural substances are seldom simple. An exception to this statement is apparently the milk fat of the monotreme which appears to be pure triolein.[1]

Simple Triglycerides. If all three of the fatty acids combined with the glycerol are the same, the fat is a simple triglyceride. Such glycerides can be readily prepared by the method of Garner,[2] who heated equivalent quantities of fatty acid and glycerol in an atmosphere of carbon dioxide at a temperature of 200° C. for six hours, during which time the esterification flask was rotated. The yield of glyceride is reported as being almost equal to the theoretical amount.

Clarkson and Malkin[3] studied the physical properties of some simple synthetic triglycerides, coming to the conclusion that simple triglycerides exist in two crystal forms which were called α- and β-, and as a glass, the γ-form. The results of their study of the melting points and X-ray spacings are shown in Fig. 25.

The β-form, the stable variety, is the only form which shows alternations in the melting point between the even- and odd-numbered-carbon-atom series. Wooley and Sandin[4] reported X-ray studies on trinondecylin which are in agreement with those of Clarkson and Malkin.

Triglycerides show a melting point which is always higher than the solidifying point. Thus the melting point of tristearin is 71.6° C., whereas it solidifies at 70° C. These two points very probably corre-

[1] H. R. Marston, Australian J. Exptl. Biol. Med. Sci. **3**, 217 (1926).
[2] T. B. Garner, J. Soc. Chem. Ind. **47**, 2791 (1928).
[3] C. E. Clarkson and T. Malkin, J.C.S. **1934**, 666.
[4] D. W. Wooley and R. B. Sandin, J.A.C.S. **57**, 1078 (1935).

spond to the two crystal modifications. In general, the melting points of the glycerides are higher than those of the contained fatty acids.

Mixed Glycerides. These glycerides contain more than one kind of fatty acid. In order to define these substances completely, the positions occupied by the fatty acids must be indicated. The two terminal linkages are α-, and the center position is known as β-. There are two kinds of mixed glycerides, symmetrical and unsymmetrical. If the fatty acids on the end groups are the same and the center one different, the glyceride is symmetrical; if one of the terminal fatty acids is different from the other two it is unsymmetrical.

The preparation of mixed glycerides is a long story. Grün,[5] in 1905, began to publish on the synthesis of the glycerides. In 1907, Grün and Schact[6] described in detail the methods of synthesis.

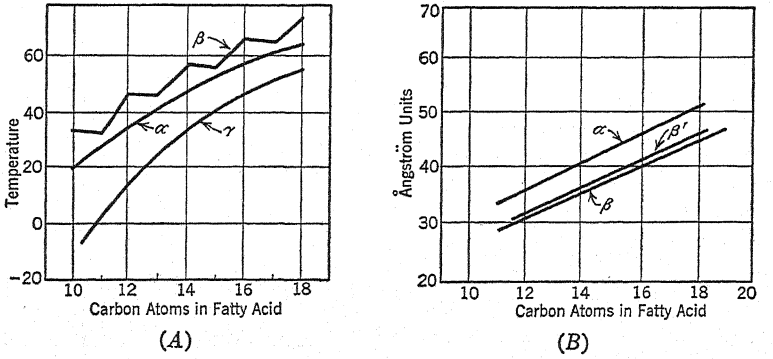


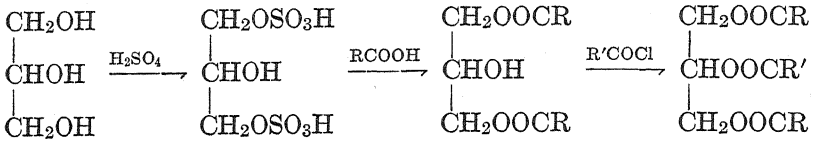
FIG. 25.—(*A*) Melting points of simple synthetic triglycerides. (*B*) Crystal spacing of simple synthetic triglycerides as determined by X-ray.

The disulfuric acid ester was first prepared by adding an excess of H_2SO_4. Then the calculated amount of free acid was added, dissolved in H_2SO_4. The mixture was kept at 70° C. for three hours. The diglyceride was cautiously washed out to remove excess acid and glycerol, then the free acid was neutralized by weak alkali and washed out. The diglyceride was purified by crystallization from chloroform and alcohol.

The purified diglyceride was converted into the triglyceride by simply adding the calculated amount of the anhydride or chloride of the acid concerned. This was supposed to go in at the β-position after being allowed to stand for several hours at 150° C.

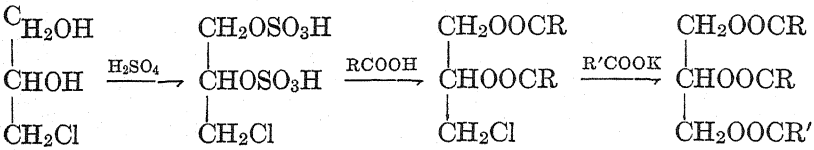
[5] A. Grün, Ber. **38**, 2284 (1905).
[6] A. Grün and P. Schact, Ber. **40**, 1792 (1907).

The reactions are



Grün's[7] method for making unsymmetrical glycerides was given in 1907.

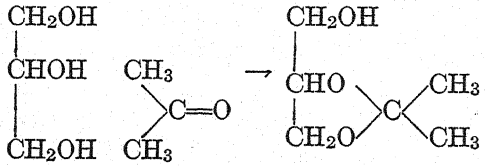
Here the α-monochlorohydrin derivative of glycerol was converted into the disulfuric acid ester, this time the α, β-ester. Addition of a fatty acid mixed with H_2SO_4 will yield an α, β-diglyceride. The second fatty acid was introduced as its potassium salt. This was allowed to stand at high temperature (150° C. or higher).



Abderhalden and Eichwald[8] busied themselves with the problem without overcoming the difficulties in Grün's work. That is, with the drastic treatment there was every likelihood of the fatty acids migrating to some other carbon atoms.

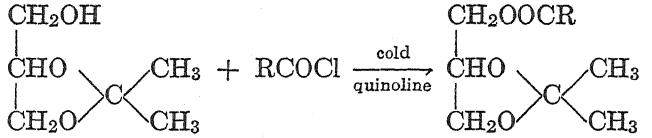
Emil Fischer[9] became interested in the problem. He stated that, at the higher temperatures used in the previous work, rearrangement might be expected to take place.

His method for making unsymmetrical triglycerides is based on the following reactions which he had previously used to acylate polyatomic alcohols. The glycerol is first condensed with acetone:

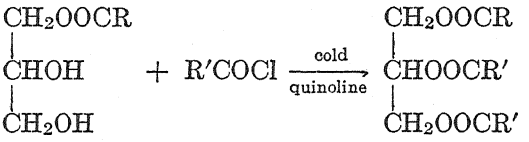


then the first fatty acid is introduced as the acid chloride in the presence of cold quinoline or pyridine to help remove the HCl that is formed:

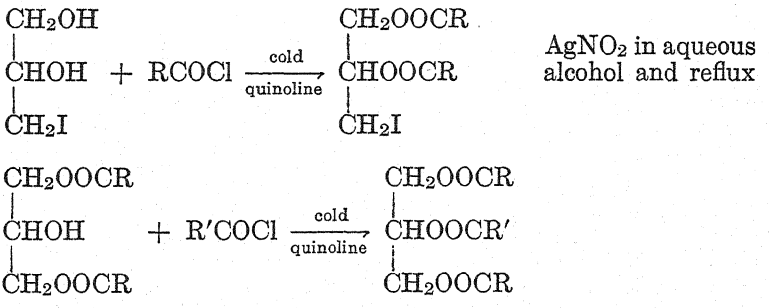
[7] A. Grün, Ber. **46**, 1792 (1907).
[8] E. Abderhalden and E. Eichwald, Ber. **47**, 1856 (1914); **48**, 1847 (1915).
[9] E. Fischer, M. Bergmann, and H. Bärwind, Ber. **53**, 1589 (1920).



The simple addition of water in the presence of ether and HCl hydrolyzes the compound and frees it from the acetone. The monoglyceride is found in the ether layer. It can be separated out and the remaining two hydroxyl groups acylated with a second acid chloride in the presence of cold quinoline or pyridine.



The symmetrical glyceride was made by starting with the α-iodohydrin glycerol and making an α, β-diglyceride, using the acid chloride in the presence of cold quinoline. The iodine was then removed with silver nitrite in the presence of alcohol by refluxing. During this process a rearrangement takes place to yield the α, α'-diglyceride, which is acylated, using quinoline as before.



In all these reactions, except during the refluxing of the nitrite where it is desired that a rearrangement take place, the temperature is kept below 30° C. At such low temperatures, rearrangement is prevented.

Recently, King *et al.*[10] have synthesized a series of symmetrical and unsymmetrical glycerides, using Fischer's reactions.

[10] H. P. Averill, J. N. Roche, and C. G. King, J.A.C.S. **51**, 866 (1929); H. P. Averill, J. N. Roche, and C. G. King, J.A.C.S. **52**, 365 (1930); H. E. Robinson, J. N. Roche, and C. G. King, J.A.C.S. **54**, 705 (1932); D. T. Jackson and C. G. King, J.A.C.S. **55**, 678 (1933); O. E. McElroy and C. G. King, J.A.C.S. **56**, 1191 (1934); B. F. Stimmel and C. G. King, J.A.C.S. **56**, 1724 (1934).

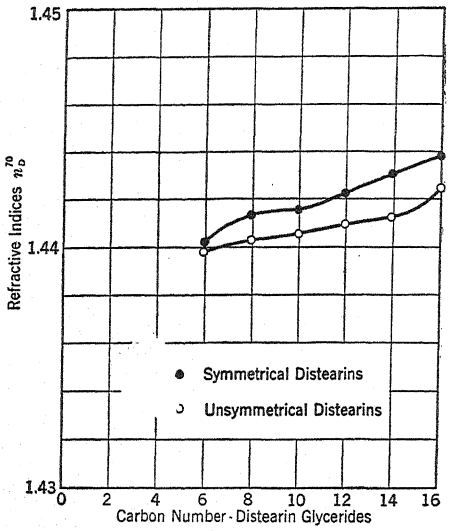


FIG. 26.—Refractive indices of the symmetrical and unsymmetrical distearins as a function of the number of carbon atoms in the odd fatty acid (70° C.).

Figs. 26 to 30 show some of their data. The carbon number refers to the number of carbon atoms in the fatty acid which is different from the other two fatty acids present in the glycerol molecule.

The symmetrical glyceride invariably has a higher melting point and higher index of refraction than the unsymmetrical triglyceride. On the other hand, the unsymmetrical glyceride has the greater solubility in all solvents, as shown in Table XIII.

There is evidence in Figs. 26 to 30 for several crystal modifications. This matter cannot be satisfactorily settled, however, until complete X-ray studies have been made. It is the understanding of the author that King and co-workers have this problem under consideration and that it will be reported on in the near future.

TABLE XIII

Compound	Solvent	Temperature ° C.	Solubility grams per 100 cc.
α-capro-α,β distearin.....	Acetone	29	39.45
β-capro-α,α distearin.....	Acetone	29	2.57
α-capro-α,β distearin.....	Alcohol	27.5	0.22
β-capro-α,α distearin.....	Alcohol	27.5	0.14
α-lauro-α,β distearin.....	Petroleum ether	27.5	38.41
β-lauro-α,α distearin.....	Petroleum ether	27.5	11.42
α-myristo-α,β distearin....	Alcohol	29	0.59
β-myristo-α,β distearin....	Alcohol	29	0.47
α-palmito-α,β distearin....	Alcohol	27.5	0.42
β-palmito-α,α distearin....	Alcohol	27.5	0.10
α-palmito-α,β distearin....	Acetone	27.5	1.82
β-palmito-α,α distearin....	Acetone	27.5	0.61

King[11] reports that none of the unsymmetrical glycerides are optically active.

Stewart[12] reports that the bitter taste of tributyrin is due to the butyric acid in the α-position and that, if butyric acid is in the β-position in a mixed glyceride containing other fatty acids which are tasteless, the resulting fat is tasteless.

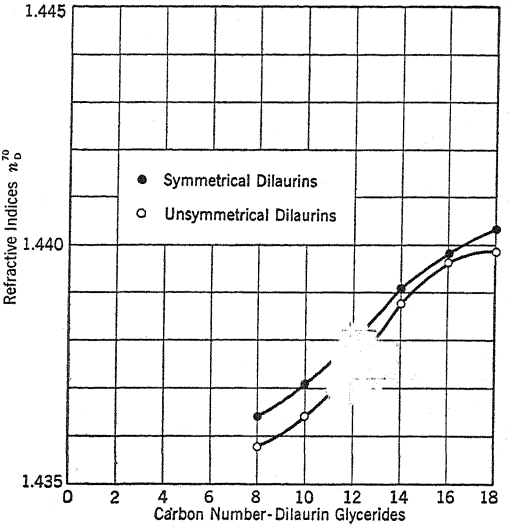


FIG. 27.—Refractive indices of the symmetrical and unsymmetrical dilaurins as a function of the number of carbon atoms in the odd fatty acid (70° C.).

HYDROLYSIS

Having considered the synthesis of fats, we now turn our attention to their hydrolysis. It seems best that the term saponification be limited to those hydrolyses in which glycerol and soaps are obtained as the ultimate products, and the general name of hydrolysis be applied to all reactions in which the fatty acids are split from the glyceride with the addition of water.

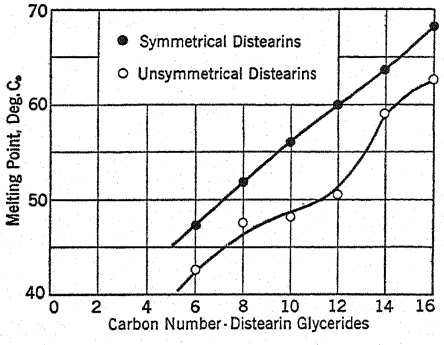


FIG. 28.—Melting points of the symmetrical and unsymmetrical distearins as a function of the number of carbon atoms in the odd fatty acid.

The hydrolysis of a fat usually does not reach completion since the reaction involved is reversible and it is often possible to synthesize a fat by use of the very agent that brought about its hydrolysis, provided that the proper medium is employed.

In general, there are six ways of hydrolyzing a fat.

1. Acid Hydrolysis. The fat is heated with dilute H_2SO_4 or HCl under pressure at temperatures exceeding 100° C. Better results are

[11] C. G. King, private communication.
[12] D. W. Stewart, Analyst **60**, 172 (1935).

obtained with H_2SO_4 than with HCl. The reaction proceeds rapidly at first and slowly towards the end. Depending upon the amount of fat present, the reaction may not reach completion for 24 hours, and, if conducted in the cold, it may take 200 or 300 days to reach equilibrium.

2. Autoclave Hydrolysis. The fat is treated with superheated steam, usually in the presence of a small amount of $Ca(OH)_2$, although water alone can be used.

3. Twitchell Method. One per cent of the Twitchell reagent (sulfobenzenstearic acid, $C_6H_4(SO_3H)\ C_{18}H_{35}O_2$) is used. The reaction seems to be purely catalytic, the Twitchell reagent remaining unchanged.

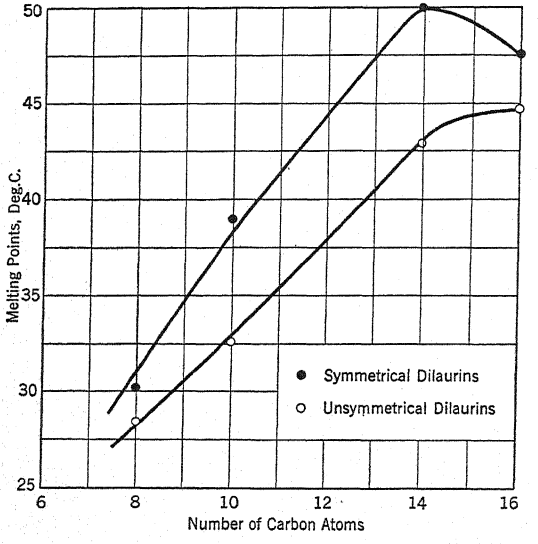


FIG. 29.—Melting points of the symmetrical and unsymmetrical dilaurins as a function of the number of carbon atoms in the odd fatty acid.

4. Saponification with Alkali. Saponification is carried out with KOH or NaOH at 100° C. It is not necessary to have the alkali in sufficient concentration to combine with all the fatty acid liberated, although, in practice, the saponification is carried out in open vessels and sufficient alkali is added not only to neutralize all the fatty acids but even to have an excess.

5. Alcoholates. The ethylate may be made either by the action of metallic sodium or potassium on the alcohol or, what is often more convenient, by the addition of the hydroxide to 95 per cent alcohol. In good work, it is well to purify the alcohol and rid it of aldehydes by treatment with freshly precipitated silver oxide and then distil the alcohol. This method of hydrolysis is often used. It is rapid; the fat and alcoholate are refluxed for about half an hour.

Bryant and Smith[13] have studied the influence of structure of both the acyl and alkoxyl portion of the ester on the rate of saponification in 0.5 *N* alkali in 90 per cent methyl alcohol. They found that alkyl substitution had a retarding effect on saponification and this retarding

[13] W. M. D. Bryant and D. M. Smith, J.A.C.S. **58**, 1014 (1936).

effect was greater the closer the substitution was to the —C(=O)—O— group. The introduction of unsaturated linkages or hydroxyls produced no appreciable hindrance, even when involving the α-position.

6. Lipase. Since lipases of certain organs, such as the stomach, decompose not only true fats but also compound lipids and various esters, the whole group is often classified as esterases, and these are subdivided into (1) lipases which act upon true fats and oils and (2) butyrases which split the esters of lower fatty acids and mono or polyvalent alcohols.

Lipases may be obtained both from plant and animal sources. The castor bean lipase is the enzyme which has been most extensively investigated in the plant kingdom. Longenecker and Haley[14] reported, recently, a study of the *Ricinus* lipase from the castor bean. They simply used the ether-extracted bean powder without additional purification. They did not determine the *p*H-activity curve but adjusted the *p*H to 4.8 with acetic acid as directed in the literature.[15] They were interested in investigating the reported specificity of *Ricinus* lipase for the oils of higher molecular weight. Although they find that, on a weight basis, considerably more of the higher-molecular-weight oils were hydrolyzed, they state that, on a molecular basis, this is not true. There has really been no extensive investigation of the plant

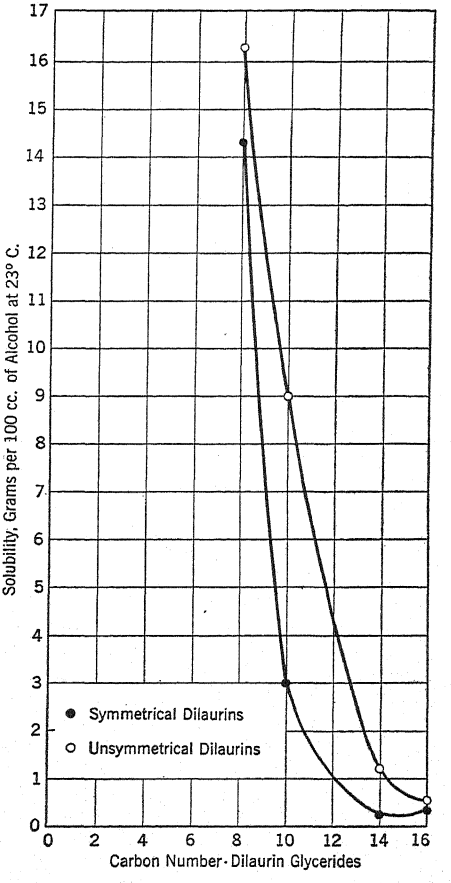


FIG. 30.—Solubility of the symmetrical and unsymmetrical dilaurins in ethyl alcohol at 23° C. as a function of the number of carbon atoms in the odd fatty acid.

[14] H. E. Longenecker and D. E. Haley, J.A.C.S. **57**, 2019 (1935).
[15] K. G. Falk, J.A.C.S. **35**, 601, 616 (1913).

lipases, and such a study is badly needed. During seed formation, the lipase content is known to decrease rather sharply as oil accumulates.[16]

In the past few years, considerable interest has been shown in the pancreatic lipase and the liver butyrase (or esterase as called by some authors). Murry and King[17] investigated the affinity of liver esterases for optically active alcohols by measuring the inhibitory power of such alcohols on the hydrolysis of ethyl butyrate or ethyl propionate by liver esterases. The levo forms of methyl-*n*-hexylcarbinol, methyl phenylcarbinol, and methyl-β-phenylethylcarbinol inhibited sheep-liver esterase about four or five times more strongly than did the dextro forms, showing that their affinity is much greater. On the other hand, the inhibition of rabbit-liver esterase by dextro and levo alcohols was practically the same.

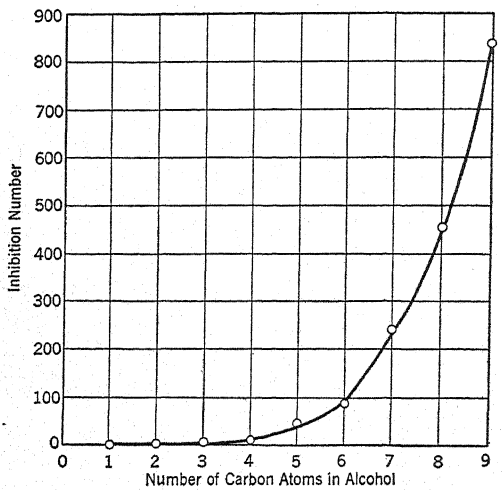


FIG. 31.—Inhibition number of saturated aliphatic alcohols on liver esterase.

Glick and King[18] studied the inhibiting effect of saturated aliphatic alcohols on liver esterase and found the higher alcohols to be much more effective inhibitors than the lower ones. In Fig. 31 the inhibition number (ratio of the number of mols of methyl alcohol needed to produce 25 per cent inhibition to the number of mols of another substance needed to produce the same inhibition under the same conditions) is plotted against the number of carbon atoms in the alcohol. This is certainly a typical capillary effect and suggests that the alcohols produce their inhibition by being adsorbed on the active spots of the enzyme. Glick and King[19] found, in general, that those substances which inhibited the esterases were activators for the lipases, and the more capillary-active a substance, the better an activator it was.

Glick and King[20] report that pancreatic lipase is either, itself, a

[16] E. R. Theis, J. S. Long, and C. E. Brown, J. Ind. Eng. Chem. **21**, 1244 (1929).
[17] D. R. P. Murry and C. G. King, Biochem. J. **24**, 190 (1930).
[18] D. Glick and C. G. King, J.B.C. **94**, 497 (1931).
[19] D. Glick and C. G. King, J.B.C. **97**, 675 (1932).
[20] D. Glick and C. G. King, J.A.C.S. **55**, 2445 (1933).

globulin or is associated with one because it has the solubilities of a globulin. It is soluble in dilute salt solutions and precipitable with 55–60 per cent ammonium sulfate, and they made use of this fact to prepare very active enzymes. Sobotka and Glick,[21] on the other hand, report the liver esterase to have the properties of an albumin. Sobotka and Glick studied the kinetics of hydrolysis with both the liver esterase and the pancreatic lipase. The kinetics of liver esterase action is orthodox and may be described simply as a linear or zero molecular reaction. The kinetics of the pancreas lipase action, however, was very complicated. They found the reaction curves for all substrates to flatten within a short time after a steep rise and follow an almost horizontal course when only a few per cent of the total possible hydrolysis had been effected. The further addition of lipase was without effect. If, however, additional substrate was added, a new rise in the reaction curve was found to be followed by a flattening out, and so on. The authors explain this peculiar behavior by assuming active and inactive spots on the enzyme, both of which adsorb or bind the substrate. The substrate bound by the inactive spots is naturally not available for hydrolysis, and it is only when new substrate is added that the active spots are effective. In other words, the enzyme itself contains an inhibitory group. This also explains how it is possible to activate lipase by adding capillary-active material. This capillary-active material is preferentially adsorbed by the inactive spots, leaving only the active spots for the substrate. Incidentally, the binding of the substrate by the inactive spots must be very strong and practically irreversible; otherwise, there would be a transfer of the substrate from the inactive to the active spots as hydrolysis proceeded.

Sobotka and Glick[22] compared the activity of liver esterase and pancreatic lipase as a function of the hydrogen-ion concentration. They found the behavior of the enzymes in this respect to be markedly influenced by the degree of purification and by the type of buffer used. This is shown in Fig. 32. Weber and King[23] found that pancreatic lipase hydrolyzes the α- and β-monolaurins, monomyristins, monopalmitins, and monostearins at approximately the same rate.

Baker and King[24] described the improved methods for the preparation and purification of the liver esterase. They confirmed the statement that it is an albumin and studied the competitive and non-competitive inhibitors. With two inhibitors of similar chemical type present

[21] H. Sobotka and D. Glick, J.B.C. **105**, 199 (1934).
[22] H. Sobotka and D. Glick, J.B.C. **105**, 221 (1934).
[23] H. H. R. Weber and C. G. King, J.B.C. **108**, 131 (1935).
[24] Zelma Baker and C. G. King, J.A.C.S. **57**, 358 (1935).

in equivalent amount, the inhibition exerted was a simple additive effect; but when the two inhibitors were of different chemical types, the inhibition exerted was greater than an additive effect, indicating their attachment to different groups on the enzyme and, hence, offsetting the declining slope of the inhibition curve, with higher concentrations.

It is suggestive that the pancreatic lipase was found to have the properties of a globulin and apparently to contain a lipid-like group which constitutes the inactive spots. It is well known that a phospholipid confers globulin-like characters on a protein. In fact, Chick has

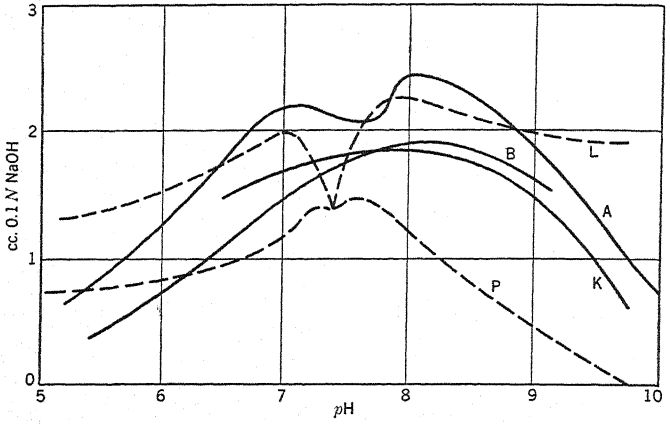


FIG. 32.—Effect of buffers on human liver esterase ——; *p*H curve of hog liver and pancreas - - - -. Substrate: 0.088 *N* methyl butyrate; 0.4 cc. of glycerol extracts in 20 cc., except that for hog liver 0.3 cc. was used. Curve *A*, buffer with diammonium phosphate (30 minutes); Curve *B*, boric acid-borax buffer (30 minutes); Curve *K*, phosphate-borate mixture (30 minutes); Curve *L*, hog liver, diammonium phosphate (15 minutes); Curve *P*, hog pancreas, diammonium phosphate (60 minutes).

even claimed that a globulin is pseudo globulin plus phospholipid, and that a globulin always contains a phospholipid. It may be that the inactive spots of the pancreatic lipase is phospholipid in nature. It is interesting, in this connection, that McGuire and Falk,[25] in a study of the lipase action of pneumococci, found that the pseudo globulin fraction of the antiserum, *i.e.*, the refined pneumococcus antibacterial preparation, upon being separated and redissolved had practically no lipase activity.

Sym[26] studied the esterification of oleic acid to glycerol in the

[25] G. McGuire and K. G. Falk, J.B.C. **105**, 669 (1934).
[26] E. A. Sym, Biochem. J. **24**, 1265 (1930).

presence of lipase from pig pancreas. He investigated the effect of the extent of surface, and his results are shown in Fig. 33.

Lipase action is important in the digestion of fats in the intestines of animals. A certain amount of hydrolysis of fats takes place in the stomach of mammals, but under most conditions it must be very small. The fat is ordinarily not emulsified, and the acidity is too high in the stomach. Only, perhaps, in the case of milk, where the fat is already emulsified and the milk protein buffers the acidity, does appreciable lipase action occur in the stomach. It is well known that a fatty meal passes through the stomach very slowly.

Two fat-splitting enzymes are present in the intestines, one being provided by the pancreatic juice and the other by the intestinal juice.

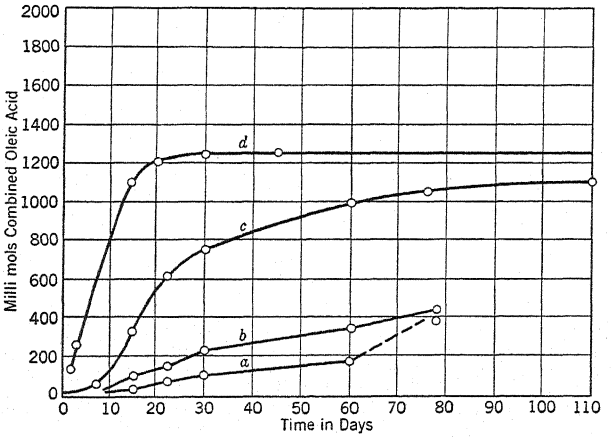


FIG. 33.—Influence of surface on velocity of esterification. (*a*) 19.5 cm.^{2} (*b*) 62.8 cm.^{2} (*c*) 399.0 cm.^{2} (*d*) 356 $\times$ 10^{3}cm.^{2}

The intestinal juice secreted during the normal process of digestion possesses a slight but definite fat-splitting action; that secreted in response to the introduction of olive oil into a fistula is more powerful in this respect. This indicates that there is a mechanism for the provision of lipase in the intestinal contents by the secretion of intestinal juice when the presence of undigested fat indicates the need for such an enzyme.

The nature of the fat ingested is important as far as its digestion is concerned. The controlling factor, here, seems to be the melting point of the fat. Tofte[27] was able to show that the hydrolysis of a fat by pancreatic lipase is primarily a function of the melting point of the fat,

[27] Finn Tofte, Biochem. Z. **272**, 308 (1934).

and the extent of unsaturation of the fat *per se* has no influence but affects the result only in so far as it lowers the melting point of the fat. Sherman[28] concludes that, if the melting point of a fat is much above the body temperature, it will not become sufficiently fluid in the intestines to be really emulsified and digested. Fig. 34 shows the data upon which Sherman based this conclusion. These results indicate that the difference in the digestibility of fats melting below 43° C. is insignificant. Those with a melting point of 50° C. or above show an appreciable loss of digestibility, while stearin melting at 60° C. shows an extreme loss of 86 to 91 per cent. It is particularly interesting to note that the addition of sufficient almond oil to lower the melting point from 60° C. to 55° C. increased the digestibility of the stearin more than 88 per cent or reduced the loss from 91 per cent to 10.6 per cent. Lard, with a melting point of 43° C., is one of the most easily and completely digested fats.

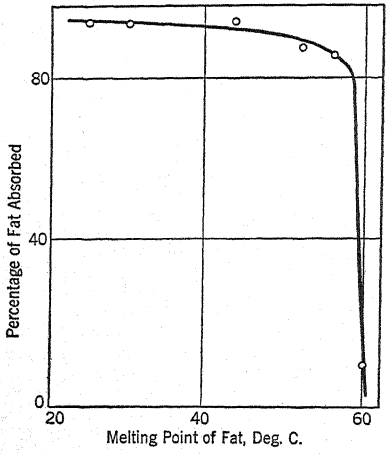


FIG. 34.—Fat absorption as a function of the melting point of the fat.

Tangl and Berend[29] and later Berend[30] have reported a dehydrogenase in the pancreas which is capable of desaturating fatty acids to a considerable extent in a fairly short time. They make the suggestion that the saturated fatty acids are desaturated in the intestines and are thus rendered diffusible and capable of being absorbed. This finding and the theory certainly need confirmation before final acceptance.

LIPID ANALYSIS

It seems useless to describe or even mention the host of analytical methods which have been proposed. Only the more important will be considered. A number of references can be consulted for detailed procedures. The recent American Chemical Society Monograph "Vegetable Fats and Oils" by Jamieson and the "Methods of Analysis" of the Association of Official Agricultural Chemists are excellent.

[28] H. C. Sherman, Food Products, Macmillan Co., New York, 1924.
[29] H. Tangl and N. Berend, Biochem. Z. **220**, 234 (1930).
[30] N. Berend, Biochem. Z. **260**, 490 (1933).

Extraction. Frequently, the lipid is combined with, or mixed rather intimately with, some material from which it must be separated before it can be analyzed. Numerous methods of extraction have been used. The selection of the method is governed partly by the nature of the material to be extracted. The A.O.A.C. expresses the lipid fraction as the "ether extract." With this method, the ground substance is continuously extracted with purified anhydrous ether for sixteen hours. Frequently, this treatment does not suffice to remove all the lipids, and other methods have been suggested. The A.O.A.C. has a tentative method for baked products. The baked product is first treated with strong ammonia and then extracted with ether. Numerous methods have been used for the extraction of plasma and tissue lipids. Man and Gildea[31] refluxed the plasma with 3 parts of alcohol and 1 part ether for one hour, after which the proteins were filtered off. Apparently, the use of alcohol is necessary to remove all the lipids, particularly the phospholipid fraction. Boyd[32] claims that, if the alcohol-ether extracts of blood are sufficiently dilute, lipids are rapidly and completely extracted, and that heat or prolonged periods of cold extraction do not increase the yield of lipids.

Lipid analytical methods may be roughly divided into three classes: (1) physical methods, (2) chemical constants, (3) separation and identification of the individual fatty acids. The physical methods include such determinations as the melting point, the solidifying point, the softening point, the transition point, X-ray analysis, and index of refraction. The use of the transition point and the melting point, as well as of X-ray analysis, has been considered under fatty acids, waxes, alcohols, and paraffins. These are extremely useful and powerful methods of attack. So far, only certain English workers have taken advantage of this procedure. The index of refraction is closely related to many of the properties of the lipid. It is to be recommended because of its simplicity.

CHEMICAL CONSTANTS

Acid value is a measure of the quantity of free fatty acid; it is defined as the number of milligrams of KOH required to neutralize the free fatty acid in 1 gram of the substance.

The saponification number is the number of milligrams of KOH required to saponify 1 gram of fat or oil. It is related to the molecular weight of the fatty acids of the oil. The higher the saponification number, the lower the molecular weight, as is shown in Table XIV.

[31] E. B. Man and E. F. Gildea, J.B.C. **99**, 43 (1932).
[32] E. M. Boyd, J.B.C. **114**, 223 (1936).

TABLE XIV

Name	Molecular Weight	Saponification Value
Tributyrin	302.2	557.0
Tricaprin	554.4	303.6
Trilaurin	638.5	263.5
Trimyristin	722.7	233.06
Tripalmitin	806.8	208.65
Tristearin	890.9	188.9
Triolein	884.8	190.2
Trilinolein	878.8	191.5
Trilinolenin	872.7	192.9

"Unsaponifiable matter" includes all those substances which are not saponified by alkali, but which are soluble in ether and petroleum ether. Such substances are the sterols, the higher alcohols, the hydrocarbons, and the ketones.

The volatile fatty acids are those acids which can be separated from the hydrolyzed fat or oil by steam distillation. With the exception of butter fat, cocoanut, palm kernel, and a few other oils, small amounts of fatty acids are obtained. Standard conditions of distillation must be rigorously adhered to in order to obtain comparable results. The volatile fatty acids consist chiefly of those in the series from butyric to lauric. The volatile fatty acids are divided into two groups, depending upon their solubility or insolubility in water.

The Reichert-Meissl value is defined as the number of cubic centimeters of 0.1 *N* KOH required to neutralize the soluble volatile fatty acid from 5 grams of fat. This number is especially useful in the characterization of butter fat because butter fat has an unusually high Reichert-Meissl value.

The Polenske number is defined as the number of cubic centimeters of 0.1 *N* KOH required to neutralize the insoluble volatile fatty acids from a 5-gram sample of fat.

The acetyl value is defined as the number of milligrams of KOH required to neutralize the acetic acid obtained by the saponification of the acetylated product. The acetylization is brought about by heating the fat with acetic anhydride whereupon the acetyl radical is substituted for the free hydroxyl groups present. The determination is, therefore, a measure of the free hydroxyl group present. Castor oil has a high acetyl value, ranging from 142 to 150. The figures for the common oils range from 2.5 to 20. It should be noted that old oils frequently

contain more or less hydrolyzed glycerides, which, having an exposed hydroxyl group, will react with acetic anhydride and give an abnormally high acetyl value.

West, Hoagland, and Curtis[33] report an improved method for the acetyl value. The reaction is carried out in pyridine, and the excess acetic anhydride is decomposed by the addition of water and titrated after sufficient butyl alcohol has been added to give a homogeneous solution. They claim that their method is applicable to free hydroxylated fatty acids. They suggest that the acetyl value be redefined as the milligrams of acetyl taken up per gram of substance.

The iodine number is the number of grams of iodine absorbed by 100 grams of fat. As the absorption of iodine alone by unsaturated acids or glycerides is exceedingly slow, it is necessary to use some halogenating agent. The two older methods, which are in common use, are the Hanus and the Wijs, which employ iodine monobromide and iodine monochloride, respectively. Both are dissolved in glacial acetic acid. The Hanus solution is somewhat more stable but usually gives values from 2 to 4 per cent lower than the Wijs method.

Recently, Yasuda[34] has adapted the Rosenmund-Kuhnhenn method, which uses pyridine sulfate dibromide as a halogenating agent, to the determination of small amounts of oil. He finds the method admirably suited for the determination of lipids in biological material. It yields almost theoretical values with cholesterol where the Hanus method fails. In general, the values obtained with oils are somewhat lower with the Rosenmund-Kuhnhenn than with the Hanus method; these lower values, however, appear to be closer to the theoretical value.

The iodine number is a measure of the degree of unsaturation of the fatty acids. Oleic acid has an iodine number of 89.93, linoleic 181.16 and linolenic 273.7. If, however, the unsaturation occurs near the carboxyl group, the iodine number is found to be much lower than the theoretical, as is shown in Table XV.

Caldwell and Piontkowski[35] have recently discussed the relation of the iodine number to the position of the double bond. There is, often, a very close relation between the iodine number and the index of refraction of a glyceride. The index of refraction is a function both of the degree of unsaturation and of the length of the carbon chain and therefore is, of course, not in general a simple function of the iodine number, but for samples of the same oil one may be considered a fair measure of the other. Peter and Kron[36] were able to formulate an

[33] E. S. West, C. L. Hoagland, and G. H. Curtis, J.B.C. **104**, 627 (1934).
[34] M. Yasuda, J.B.C. **94**, 401 (1931).
[35] B. P. Caldwell and F. A. Piontkowski, J.A.C.S. **56**, 2086 (1934).
[36] Peter and Kron, Milch Forschungen, **14**, 378 (1932).

TABLE XV

Acid	Formula	Theoretical Iodine No.	Actual as Determined
Crotonic.....	$CH_3CH{=}CHCOOH$	295	25
Fumaric.....	$\begin{matrix} HCCOOH \\ \| \\ HOOCCH \end{matrix}$	219	6.0
Maleic.....	$\begin{matrix} HCCOOH \\ \| \\ HCCOOH \end{matrix}$	219	6.0
Cinnamic....	$C_6H_5CH{=}CHCOOH$	170.9	15.3
2–3 Oleic....	$CH_3(CH_2)_{14}CH{=}CHCOOH$	90.07	9.04
3–4 Oleic....	$CH_3(CH_2)_{13}CH{=}CHCH_2COOH$	90.07	16.27
4–5 Oleic....	$CH_3(CH_2)_{12}CH{=}CH(CH_2)_2COOH$	90.07	26.9

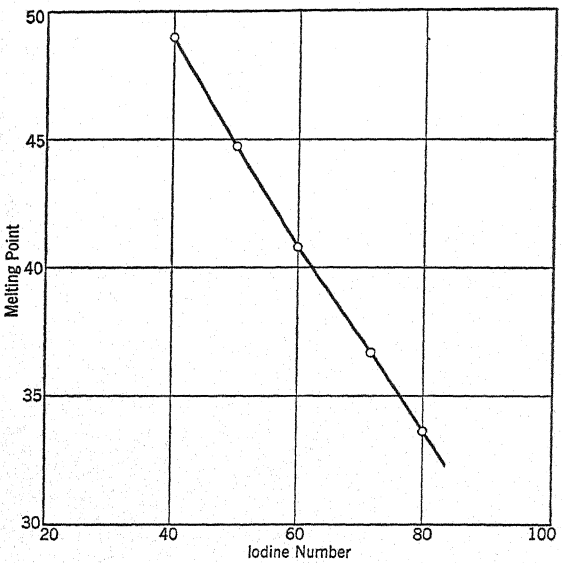


FIG. 35.—Melting point of hydrogenated cottonseed oil as a function of the iodine number

empirical relation between the index of refraction and the iodine number of butter fats.

Figs. 35, 36, and 37 show how certain properties of hydrogenated cottonseed oil vary with the degree of unsaturation.[37] These are not natural oils, but the same tendency is no doubt shown by the natural oils although a less smooth relation is to be expected.

The thiocyanogen number was developed by Kaufmann and his associates.[38] It is based on the fact that under proper conditions the radical thiocyanogen (CNS) will add at the

[37] J. J. Sudborough, H. E. Watson, and D. Y. Athawale, J. Ind. Inst. Sci. **5**, 47, (1922).

[38] H. P. Kaufmann, Analyst **51**, 157 (1926).

double bond of a fatty acid in the same way that iodine does. It helps to characterize the kind of unsaturation because thiocyanogen adds at only one of the double bonds of linoleic and at only two of linolenic. The thiocyanogen number is expressed in terms of iodine absorbed.

Acid	Iodine Number	Thiocyanogen Number
Oleic.....	89.9	89.9
Linoleic.....	181.1	90.5
Linolenic.....	273.7	182.5

It is possible, by running iodine numbers and thiocyanogen numbers as well as the total saturated acids, to calculate, by use of simultaneous equations, the percentage of each of the three common unsaturated acids present. Often such a calculation is not very successful. The method does, however, give one an excellent idea of the kind of unsaturation.

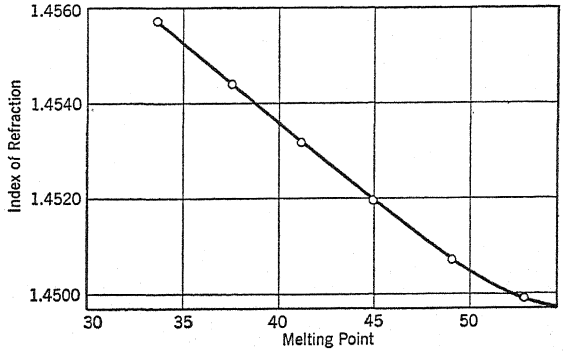


FIG. 36.—Index of refraction of hydrogenated cottonseed oil as a function of the melting point.

Bloor[39] developed an extremely important method for the determination of lipids with special reference to biological materials. He modified Bang's oxidative method and placed it on a workable basis. Bloor's method consists, essentially, of a wet combustion of the lipids by means of potassium dichromate and sulfuric acid. The lipid is supposed to be burned quantitatively to carbon dioxide and water. It is possible to calculate the chemical factors between the dichromate used and the lipid burned. For example,

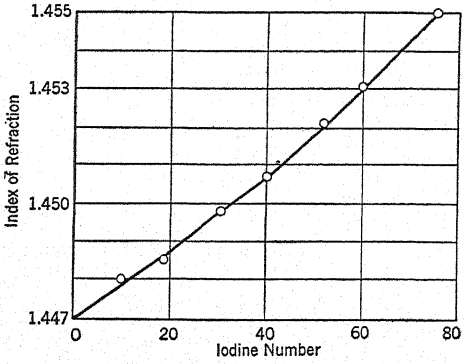


FIG. 37.—Index of refraction of hydrogenated cottonseed oil as a function of the iodine number.

[39] W. R. Bloor, J.B.C. 77, 53 (1928).

1 mg. of the following fatty acids should require the indicated number of cubic centimeters of 0.1 *N* dichromate:

Palmitic acid	3.59
Stearic acid	3.66
Oleic acid	3.61
Cholesterol	3.92

Actually, the presence of a small amount of silver dichromate was found to facilitate the oxidation greatly. It was necessary to heat the reacting mixtures at a specified temperature (124° C.) for a given time (twenty minutes) in order to obtain good checks. Cholesterol is estimated separately and taken into consideration when the fats are estimated. Bloor[40] has since adapted this method to the determination of the phospholipids. Some workers feel that it is better to estimate the phospholipid by a phosphorus determination, and they apparently do not believe that it is possible to obtain a complete precipitation of small quantities of phospholipid from acetone with $MgCl_2$ as is required by the Bloor method.

Van Slyke, Page, and Kirk[41] have turned the Bloor method into a gasometric one and measure the carbon dioxide evolved in the liquid combustion with dichromate. Page *et al.*[42] have since applied this method to an extensive study of the plasma lipids. There is a real need for a simple method to differentiate between the phospholipids present. It is very unsatisfactory to determine them as total phospholipid. Lecithin and cephalin probably have very different physiological functions. In a determination of the amino nitrogen by the Van Slyke method, the unsaturated fatty acids must be removed because they absorb oxygen from the nitrous oxides and so contribute to the amino nitrogen.

Allen[43] has developed a simple and accurate volumetric method for the determination of fat in blood plasma. It is not necessary to extract the lipids from the plasma. His method is a modified form of that proposed by Petersen and Herreid[44] for estimating the fat content of buttermilk.

Wilson and Hansen[45] have examined the plasma lipids by extracting the lipids, saponifying, regenerating the fatty acids, titrating them, and determining their iodine number.

[40] W. R. Bloor, J.B.C. **82**, 273 (1929).
[41] D. D. Van Slyke, I. H. Page, and E. Kirk, J.B.C. **102**, 635 (1933).
[42] I. H. Page, E. Kirk, W. H. Lewis, W. R. Thompson, and D. D. Van Slyke, J.B.C. **111**, 613 (1935).
[43] N. N. Allen, Proc. Soc. Exptl. Biol. Med. **31**, 991 (1934).
[44] Wm. E. Petersen and E. O. Herreid, Minn. Agr. Exp. Sta. Bull. **63** (1929).
[45] W. R. Wilson and A. E. Hansen, J.B.C. **112**, 457 (1936).

SEPARATION AND IDENTIFICATION OF INDIVIDUAL FATTY ACIDS

The saturated and unsaturated acids can be separated by the Twitchell method,[46] which consists in forming the lead salt of the fatty acids and treating this with hot alcohol. The lead salt of the unsaturated acids and of the shorter-chained acids are soluble and those of the higher saturated members are insoluble.

The fatty acids can be separated from each other by a careful fractional distillation of the methyl esters. Hilditch uses this method in conjunction with the lead salt method to obtain fractions which contain not more than two fatty acids.

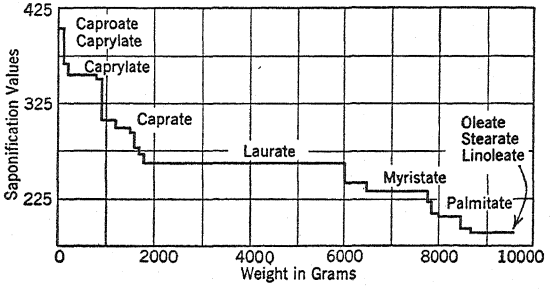


FIG. 38.—Fractional distillation of the methyl esters from cocoanut oil

Lepkovsky, Feskov, and Evans[47] have developed a fractionating column for the separation of fatty acids. They found that it is easier to separate the methyl esters than to separate the free fatty acids because the methyl esters have a lower boiling point and also the fatty acids tend to associate, making their separation more difficult. They analyzed cocoanut oil by a fractional distillation of the methyl esters. Fig. 38 shows their results.

When fats or oils are dissolved in chloroform, ether, or other solvents, and treated with bromine, the bromine addition compounds of the unsaturated acids are produced. Oleic acid yields the dibromostearic acid, linoleic the tetrabrom, and linolenic the hexabromide. The dibromides and tetrabromides are soluble in ether; the hexa and octobromides of stearic acid are almost insoluble in cold ether. This method is often used to prepare the unsaturated acids. The bromide is separated and purified, and the corresponding unsaturated fatty acid is regenerated by treating the bromide with zinc and hydrochloric acid.

Hilditch's Method. In addition to the above methods, Hilditch and his associates have perfected and developed new methods of their own and have published a long series of papers dealing with the analysis of fats. (A brief review of their work is to be found in J. Chem. Ind. 54,

[46] E. Twitchell, J. Ind. Eng. Chem. **13**, 806 (1921).
[47] S. Lepkovsky, G. V. Feskov, and H. M. Evans, J.A.C.S. **58**, 978 (1936).

139–45, 163–7, 184–9 [1935].) They have taken advantage of the older methods for the analysis, separation, and identification of the individual fatty acids. They have first separated, as completely as possible, fatty acids on the basis of solubility, *i.e.*, lead salts. Then they have subjected them to fractional distillation as the methyl esters. The novel feature of their method consists, however, in determining the amount of completely saturated glyceride and the unsaturated glyceride by oxidation with $KMnO_4$ in acetone. The unsaturated fatty acid is oxidized and split at the double bond. The glyceride is not hydrolyzed by this treatment, and the sodium salt of the exposed carboxyl group is formed; this renders the fat insoluble in cold ether and soluble in water. Then, with a knowledge of the fatty acids present, it is possible to work out the distribution of the fatty acids in the glycerides with some accuracy.

Their procedure is essentially as follows:

1. Remove free fatty acids by washing with dilute alkali.

2. Dissolve fat in acetone to make approximately a 10 per cent solution, and add powdered $KMnO_4$, a little at a time, to 25 per cent, reflux for four hours, and then let stand overnight.

3. Boil the crude oxidation product with a slight excess of Na_2CO_3 until a clear layer of neutral fat separates out.

4. Separate the layer of fat from the water phase.

5. Wash the fat until free from acid.

6. Add the washings from step 5 to the water phase from 4, and then extract the combined solution with ether. Evaporate the ether from the extract and add the residue to number 5. In this manner the saturated glycerides may be isolated, inasmuch as the unaltered fat is insoluble in water, and the sodium salts of the oxidation products are insoluble in ether.

In addition to these methods, Hilditch[48] has recently suggested that progressive hydrogenation can be of great value in fat analysis. It is known that the linoleic acid will be first reduced to the oleic acid before an appreciable amount of the oleic acid is reduced to stearic acid. It is also known that the unsaturated acids in the α-position on the glycerol are preferentially saturated before the unsaturated acids in the β-position. These facts, taken in conjunction with determinations of other fat constants, yield a good idea of the constitution of the fat.

Hilditch maintains that the lead salt-methyl-ester determination gives results that are accurate within 1 per cent for the major-component fatty acids (fatty acids present to 5 to 10 per cent and upward). It is possible to check the accuracy of his method to some extent by hydro-

[48] T. P. Hilditch and W. J. Stainsby, Biochem. J. **29**, 90 (1935).

genation, and this he has done, as shown in Table XVI. Further results of Hilditch's study will be considered under fat storage.

TABLE XVI

PALM OIL FATTY ACIDS (MOL PER CENT)

	Original		After Hydrogenation	
Myristic.....	1.9		0.7	
Palmitic.....	34.3		35.5	
Stearic.....	5.3	63.8	62.7	63.8
Oleic.....	50.6		1.1	
Linoleic.....	7.9		0.0	

NATURAL FATS AND OILS

Every species of plant and animal has its own particular kind of fat, and the kind of fat in each species varies with the age of the organism and can be profoundly affected by pathological changes. The type of fat in different organs of the same animal or plant also shows wide variations. Temperature of the environment and, with animals, the diet, have considerable influence on fat storage. No attempt will be made to describe or even mention the thousands of naturally occurring fats. We will content ourselves with a general discussion of the effect of species, age, temperature, and diet, and a short discussion of lipid pathology.

There is a large amount of rather miscellaneous information relative to fat storage in many species both of animals and plants. The only systematic and thoroughgoing investigation of which the author is aware, however, is that of Hilditch and co-workers. Reference has already been made to their analytical technique, which consists essentially of three procedures: (1) separation of the completely saturated triglyceride by oxidation of the unsaturated fatty acids with $KMnO_4$; (2) separation and identification of the individual fatty acids by a lead salt separation followed by fractional distillation of the methyl esters; (3) study of the fat with progressive hydrogenation. As Hilditch and co-workers have published a long series of papers, no attempt will be made to refer to them separately, but a résumé will be given of the more important results. The papers can for the most part be found in the Journal of the Chemical Society (London), the Biochemical Journal, and the Journal of the Society of Chemical Industry.[49]

Hilditch defines a major-component fatty acid as one which makes up 5 to 10 per cent of the total fatty acid present. Table XVII shows, in a general way, the major-component fatty acids of some common fats.

[49] For a recent review of their work, see J. Soc. Chem. Ind. 54, 139–45, 163–7, 184–9 (1935).

TABLE XVII

Fat	Saturated	Unsaturated
Seed fats of the cocoa butter	C_{16}, C_{18}	C_{18}
Palm oil, tallow, lard and many vegetable oils	$(C_{14})C_{16}$, C_{18}	C_{18}
Seed fats of the palmae	C_8, C_{10}, C_{12}, C_{14}, C_{16}	C_{18}
Milk fats	C_4, $C_6(C_8, C_{10}, C_{12})C_{14}$, C_{16}, C_{18}	C_{18}
Marine fats	$(C_{14})C_{16}$, (C_{18})	$(C_{14})C_{16}$, C_{18}, C_{20}, $C_{22}(C_{24})$

The body fats of land animals, like many of the plant fats, have only three major-component fatty acids, palmitic, oleic, and linoleic, except that in certain groups (possibly only in herbivorous animals) stearic acid is also a major-component acid.

Palmitic acid to 25–30 per cent seems very characteristic of land animals. The remaining 70 per cent of the fatty acid may be wholly unsaturated or may contain relatively large amounts of stearic acid. There is apparently a balance between the stearic acid and oleic acid, and, on the basis of his hydrogenation studies on triglycerides, Hilditch makes the suggestion that, since the variation of the total saturated triglyceride with the percentage of saturated fatty acid is the same in the pig in an *in vivo* experiment as with catalytic hydrogenation *in vitro*, the hydrogenation of the fatty acids in the pig takes place in the tissue while the fatty acids are combined to the glycerol molecule. It is a question, however, if the balance between the oleic and stearic acids is not dependent more upon the ratio of the fatty acids ingested than upon a hydrogenation-dehydrogenation reaction in the tissue, and it may be as well or better argued that oleic and stearic acids are equivalent as far as the α, α' positions of the glycerol molecule are concerned.

Rat fat presents a curious situation. While rat fat is to a considerable extent unsaturated, its unsaturation is due largely to oleic acid; linoleic acid is present to the extent of 6 per cent or less and is very sensitive to diet. If the animal is fed on a diet lacking in linoleic acid that acid is present in the resulting rat fat in very small amounts or may disappear entirely. Drummond found that, under such conditions, the liver still contained linoleic acid but the other tissues were free of the acid. This is in excellent agreement with the findings of Burr and Burr relative to the essential fatty acids. It seems that man and the rat are two of the few completely omnivorous animals, and, owing to this similarity in

feeding habits, their body fats may be similar. Hilditch has not reported on human fats, and in spite of the immense amount of work on the lipids of the human body, we still have no systematic study of the composition and constitution of the human triglycerides.

Hilditch, on the basis of his study, has made certain generalizations. To quote:

"The structure of glycerides seems to be determined solely by their place of origin in the plant or animal and to be of two sharply defined kinds.

"(*a*) Glycerides in which the fatty acid molecules are woven into combination with glycerol in such a way that maximum even distribution of the fatty acid amongst the glycerol molecules has taken place. So far, this has only been observed in vegetable seed fats but within this category it appears to be the rule almost without exception.

"(*b*) Glycerides in which the fatty acids are thrown into combination with glycerol in a more chance fashion.

"Animal body and milk fat, pericarp or fruit flesh oils of plants and artificially prepared fats made by heating fatty acids and glycerol together fall into this class. There is a higher per cent of completely saturated and completely unsaturated glycerol molecules."

In general, animal fat is of two kinds: (1) marine, with a complex mixture of fatty acids varying considerably in molecular size but for the most part unsaturated; and (2) the much simpler land-animal type. This second class is again loosely of two kinds: (*a*) depot fat of rodents and birds with 25–30 per cent palmitic acid, fatty acids being evenly distributed as with plants; and (*b*) the depot fat of pig, ox, sheep, and other herbivorous animals, in which stearic acid has assumed more important proportions and the amount of completely saturated glyceride is much greater.

The composition of the fat of frogs (amphibia) was found to fall between that of the fat of marine and land animals.

Fig. 39 (Hilditch) shows the relation between the saturated fatty acid and the completely saturated glyceride present in certain animal and plant fats.

Lovern[50] has recently published a series of papers dealing with the body fat of marine and fresh-water fish. He finds that the fat from fresh-water fish forms a class distinct from the fats of marine species. The difference has been defined as an increased proportion of C_{16} and C_{18} unsaturated acids and decreased C_{20} and especially C_{22} acids in the fresh-water-fish fat as contrasted with the fats from marine species. Fish fat seems to depend upon the diet, its character resembling that

[50] J. A. Lovern, Biochem. J. **29**, 847 (1935); **29**, 1894 (1935); etc.

of the ingested fat. On the other hand, Lovern was able to demonstrate that the carp is able to synthesize fat from carbohydrate. Lovern found that the fat of the porpoise and dolphin contains considerable quantities of isovaleric acid. This is most unusual.

Collins[51] has investigated the larva fat of the beetle *Pachymerus dactris* Linn. and compared it with kernel fat of the *Manicaria saccifera*, upon which it feeds. It appears that the lower-molecular-weight fatty acids are present in the larva fat in only about half the amount in which they occur in the kernel fat, while oleic and linoleic acids probably form about 40 per cent of the mixed acids in the larva fat, as compared with only 11 per cent in the kernel fat.

On the whole, it seems likely that insects in larval as well as in the mature state lay down fat somewhat similar in type to the fats produced by mammals, and can assimilate fat present in their diet and also synthesize it from other food constituents.

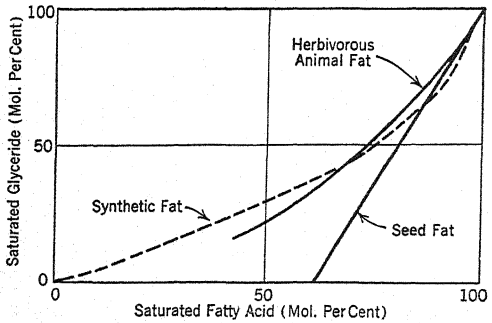


FIG. 39.—Relation between the saturated fatty acid and the completely saturated glyceride present in certain animal and plant fats.

Fats are found in practically all plant parts. The extent to which they occur in the various organs of the same plant as well as in different species varies greatly. The seeds and fruits usually contain most of the fat found in plants, and they have been extensively studied. In some seeds, as the cereals, the fats are confined almost entirely to the embryo. The quantity and quality of the oil vary considerably in the same species under different conditions so that conditions under which data were obtained must be considered. There are about 200 species of plants which are capable of furnishing sufficient oil to be of commercial interest. Some seeds may be very high in oil, for example, the cocoanut has 65 per cent oil; other seeds such as those of rice may have as little as 2 per cent. The unsaponifiable matter in seeds is usually low, amounting to only 0.2 to 0.8 per cent, whereas that of the stems and leaves is from 16 to 25 per cent.

As mentioned above, the vegetable fats are produced largely within the seed, but in others, fats are also laid down in the pericarp, receptacle, or other parts of the fruit-coat which may surround the endosperm.

[51] G. Collins, Biochem. J. 27, 1373 (1933).

Fruit-coat Fats. Palm and olive oil, and Chinese (*Stillingia*) tallow are examples of this class. Fruit-coat fats vary from very hard, tallowy fats to drying oils, but the differences in their properties depend almost entirely on the widely varying relative amounts of palmitic, oleic, and linoleic acids. There is apparently no connection between the fruit-coat fat and seed fats from the same plant.

The fruit-coat fats seem to be of the "evenly distributed" type. Thus, palm oil contains almost 90 per cent of mixed dipalmito oleins and palmito dioleins. The simple triglyceride, tripalmitin, is, however, sometimes present in fruit-coat fats.

Seed Fats. In endosperm and embryo fats there is an even more decided tendency towards the formation of mixed glycerides than in the fruit-coat fats. Palmitic, oleic, and linoleic acids are usually the major-component fatty acids. However, other fatty acids do occur. For example, the unsaturated erucic acid is present up to 40 to 50 per cent in all cruciferous seed fats; an isomer of oleic acid, the $\Delta 6,7$ octadecenoic or petroselinic acid, is found in the seeds of the Umbelliferae (parsley, celery, etc.). Arachidic and lignoceric acids occur in considerable amounts in the family Sapindaceae; stearic acid is present to any considerable extent only in the seed fat of a few tropical families. Other tropical plants tend to produce myristic and lauric acids.

Lovern[52] found the fat from algae to fall into groups agreeing with their botanical relationships, and no difference between the corresponding fresh- and salt-water forms could be detected. The fats of all green algae, the pond weed, and the diatom are of a type very similar in many respects to fresh-water animal fats.

Changes in Lipids During Seed Formation. As the seeds develop, the lipid content gradually increases, at the same time the degree of unsaturation increases. The oil occurs first as fine droplets increasing in size with age so that in the old cells large masses or drops of oil can be found. The lipids, during ripening, apparently form the continuous phase. The reverse of this occurs during germination. Condensation attends the storage of the fats while dispersion accompanies their digestion.

Theis, Long, and Brown[53] studied the lipids of flaxseed as well as the lipase activity of these seeds as a function of time. Their results are shown in Fig. 40.

Fig. 41 shows the unsaturation as a function of time from flowering.[54] Environmental factors are important in so far as the kind and quan-

[52] J. A. Lovern, Biochem. J. **30**, 387 (1936).
[53] E. R. Theis, J. S. Long, and C. E. Brown, J. Ind. Eng. Chem. **21**, 1244 (1929).
[54] I. J. Johnson, J. Agr. Research, **45**, 239 (1932).

tity of oil of plants are concerned. It is a general rule, for example, that tropical-plant lipids are more saturated than temperate-zone ones. Stark[55] studied the effect of different fertilizers on the oil content of soy beans. Applications of limestone and organic matter were found to increase the percentage of protein and decrease the percentage of oil. Rock phosphate increased this effect. Potassium, in addition to rock phosphate, limestone, and organic residues, resulted in a decrease in the amount of protein and increase in the oil. No consistent relation was found between the iodine number and soil treatment. In general, conditions which tend to increase the protein send the oil down, and *vice versa*.

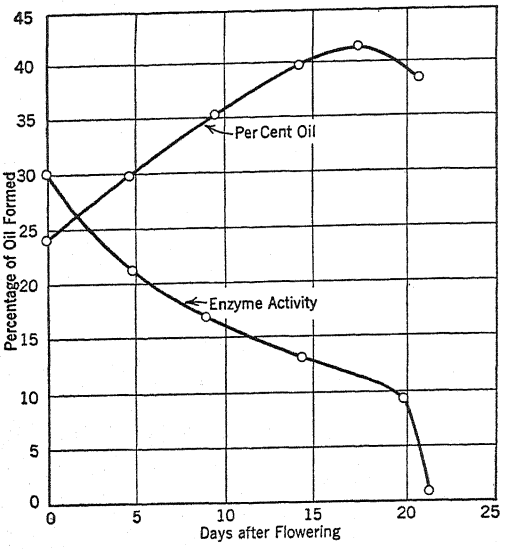


FIG. 40.—Lipase activity and oil content of developing flaxseed

Johnson[56] found a negative correlation between the quantity of oil in flaxseed and the iodine number of the oil. Arny[57] reports that unsaturation is a definite genetic factor in flaxseed and is closely associated with yellow seeds. Brown seeds yield oil with less unsaturation.

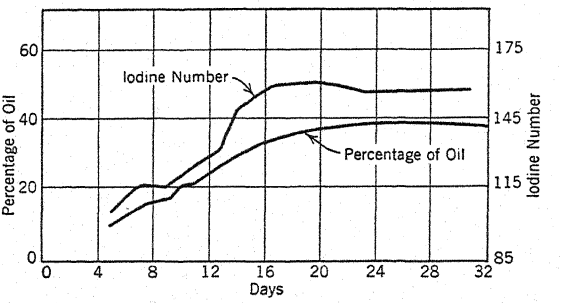


FIG. 41.—Iodine number and oil content of developing flaxseed.

During germination, seeds apparently reverse the changes described for seed formation.[58] The unsaturated fatty acids disappear first,

[55] R. W. Stark, J. Am. Soc. Agron. **16**, 636 (1924).
[56] I. J. Johnson, J. Am. Soc. Agron. **24**, 537 (1932).
[57] A. C. Arny, private communication.
[58] E. C. Miller, Ann. Botany **26**, 889 (1912).

leaving the oil more saturated. The quantity of oil decreases and the carbohydrates increase. Hugo Magistris[59] discusses the lipids of fungi and bacteria. Yeast contains a great variety of sterols. Anderson and associates have in recent years conducted extensive investigations of the lipids of bacteria.

Diet and Animal Fat. The type and extent of diet affect fat storage considerably. It is well known, for example, that the feeding of carbohydrates tends to give rise to a hard fat. Table XVIII shows the effect of the various diets on the back fat of hogs.[60]

TABLE XVIII

COMPOSITION OF BACK FAT FROM HOGS ON DIFFERENT FEEDS

Acids in Fat	Brewer's Rice	Corn	Peanuts	Soybeans
Oleic	55.9	52.0	54.3	38.7
Linoleic	1.2	6.7	18.6	30.5
Linolenic	0.0	0.0	0.0	0.2
Arachidonic	0.02	0.06	0.12	0.08
Myristic	1.7	0.6	0.4	0.7
Palmitic	25.2	24.1	14.8	16.6
Stearic	11.6	12.2	7.1	9.0
Arachidic	0.0	0.0	0.2	0.0

From Table XVIII it will be noted that the type of ration fed has a pronounced influence upon the composition of the lard. It is believed that oleic, palmitic, and stearic acids are formed from carbohydrates.

Recently, Schoenheimer and Rittenberg[61] have indicated that depot fat undergoes rather active metabolism. They brought the body fluids of mice on a bread diet to an increased concentration of heavy water (D_2O). The fatty acids (both saturated and unsaturated) of the animals were found to contain considerable amounts of "stable" deuterium, a maximum being reached after six to eight days. The unsaturated fraction, on oxidation, yielded azelaic acid containing deuterium in the same concentration as in the original unsaturated acids. They point out that there are probably no fatty acids which contain double bonds in the fragment represented by the azelaic acid and, therefore, the deuterium could not have entered it by saturation of double bonds present in positions in which they exist in unsaturated acids in the tissue. Since the mice did not change their weight, the observed synthesis must have been accompanied by a simultaneous breakdown. This metabolism

[59] H. Magistris, Ergebnisse Physiol. **31**, 351 (1931).
[60] N. R. Ellis and H. S. Isbell, J.B.C. **69**, 239 (1926).
[61] R. Schoenheimer and D. Rittenberg, J.B.C. **114**, 381 (1936).

of the fatty acids was confirmed by feeding of deuterium-rich fatty acids and noting the time taken for the disappearance of deuterium from the tissue fats after the feeding of these fatty acids was discontinued. The rate of disappearance was about the same as that of the formation in the first experiment. Cholesterol behaved in a similar manner. Some of their data are shown in Fig. 42.

The question of diet is of considerable interest to hog raisers who sell their products to lard makers, who, in turn, are interested in the "shortening" quality of the fat. It seems that the term "shortening agent" as applied to lard is an appropriate one. Apparently the fat spreads over the surface of the protein and starch and prevents them from forming tough, stringlike material. It "shortens" the fibers and gives to the baked product a smaller breaking strength and a smaller crushing strength. The action of shortening as examined under the microscope with suitable dyes is very similar to that of lubricants. The better the lubricating power of the shortening, the shorter the product and, accordingly, less shortening will be required to produce a tender product.

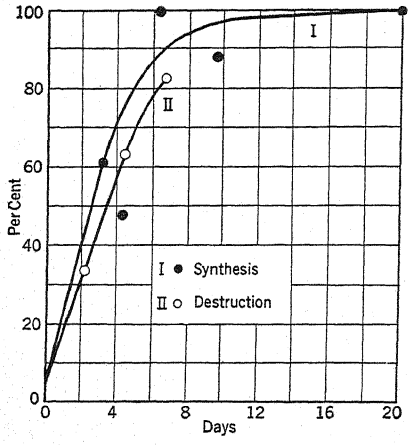


FIG. 42.—Rate of synthesis and destruction of body fat of mice.

Temperature. The temperature at which an animal is kept has an important effect on the degree of saturation of the fat stored by an animal. The animal attempts, apparently, to store the highest-melting-point fat (most saturated) that can be safely stored. Whether this is in order to obtain the higher fuel value of the more saturated fat or because it is chemically easier to produce a saturated fat than an unsaturated one is not known. In the experiments of Henriques and Hansen three pigs from the same litter were kept at different temperatures, the first at 30° C. to 35° C., the second at 0° C., and the third at 0° C. but covered and kept warm with a sheepskin coat. After two months, the pigs were killed and the fat analyzed. It was found that the fat of the pig kept at 0° C. without any cover showed the highest iodine number (72.3). The pig kept at 30° C. to 35° C. had deposited fat with an iodine number of 69.4, whereas the clothed pig kept at 0° C. had the most saturated fat with an iodine number of 67.0.

Location. The amount and character of fat vary from organ to organ. A typical distribution of the reserve fat of the pig is as follows:

TABLE XIX

	Per Cent of Total Fat
Subcutaneous	50
Genital	20
Perinephric	12
Mesenteric	10
Intermuscular	5

Table XX shows how the degree of unsaturation of depot fat varies in different parts of the body of normal men.[62]

TABLE XX

Organ Fat	Iodine Number
Panniculus adiposus abdominalis	70
Omental	63.5
Perinephric	63.0
Epicardial	63.5
Liver	127.0

Fat Pathology. The storage of fat may be tremendously disturbed by certain pathological conditions. Among these are diabetes, which we have considered under fatty acids, the excretory diseases, obesity, fatty degeneration, fat necrosis, and, in dead bodies, adipocere.

The fat-excretory diseases are sprue and celiac. Non-tropical sprue and tropical sprue are now usually considered to be the same disease. Celiac is regarded by the American workers as the infant form of sprue. In these three conditions, the stools may contain as high as 70 per cent material of a lipid character (normal stool is about 20 per cent fat). Very little neutral fat is present, most of it being free fatty acid and soap. The unsaponifiable matter is high. It is believed that this fat is excreted into the intestines and the condition is definitely not due to lack of ability on the part of the organism to digest and absorb fat. Accompanying this disturbance in the lipid picture is a blood calcium deficiency, patients occasionally going into tetany as the result of low calcium. There is, however, no abnormality in the phosphorus. The symptoms resemble those for pernicious anemia. The disease is probably congenital but it responds to dietary treatment.

In order to understand the pathological excretion of fat, one should consider the normal excretion. Most of the work on fat excretion has been done by W. M. Sperry and co-workers. They found (*a*) that there is

[62] D. P. Cuthbertson and S. L. Tompsett, Biochem. J. 27, 1103 (1933).

a definite endogenous excretion of fatty material in dogs; (*b*) that bile is not the source of this lipid; (*c*) that the bacteria found in the intestinal epithelium is not significant as a source of the excreted lipid; (*d*) that desquamation of intestinal epithelium is not significant as a source of the excreted lipids; (*e*) that the sterol portion of the excretion is passed out mainly into the large bowels; and (*f*) that probably a fairly heavy excretion of fat takes place in the small intestines and a large amount of this fat is reabsorbed in the large intestines.

Obesity may be due to over-eating and lack of exercise or to some glandular upset. It is frequently bettered by administering thyroxine. Recently, dinitrophenol has been used; apparently it facilitates the burning of fat. It should be mentioned that the use of dinitrophenol is extremely dangerous. In fact, both substances mentioned should be used only on the advice and under the supervision of a competent physician. It is a common observation that women are more subject to obesity than men.

Fatty degeneration represents either or both of two conditions. (1) It may result from an increase in the normal quantity of fat in an organ undergoing parenchymatous degeneration, through an infiltration of fat from the outside; this is particularly true of the liver. (2) There may be no increase in the total amount of fat, but the invisible fat becomes visible through autolysis, or hydration changes in the cell protein. The normal heart contains about 15 per cent fat; the heart undergoing degeneration may contain 25 per cent or more of fat. It is known that feeding large quantities of cholesterol leads to fat accumulation in the liver. This accumulation may be prevented by feeding lecithin, choline, or betaine.

Sobotka *et al.*[63] have studied the lipid distribution during certain rather rare lipid diseases with the idea of gaining some insight into lipid metabolism because, as they point out, the accumulation of a certain lipid fraction probably means that this fraction is a normal intermediate in lipid metabolism but owing to the absence of the proper enzymes the accumulated lipid is not further metabolized. They consider three pathological conditions and report on two of them.

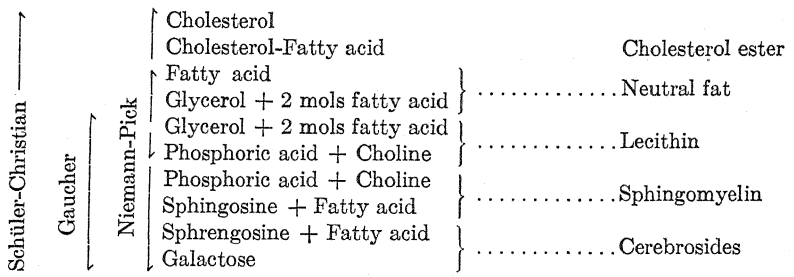
1. Gaucher's splenomegaly, characterized by large deposits of the cerebroside, kerasin, in the spleen and liver.

2. Niemann-Pick's disease, in which the phospholipid and cholesterol contents of the viscera, the bone marrow, and the brain are increased at the expense of the neutral fat.

3. Schüler-Christian's disease, in which there is a replacement of bone tissue by cholesterol deposits.

[63] H. Sobotka, D. Glick, M. Reiner, and L. Tachman, Biochem. J. 27, 2031 (1933).

These conditions which lead to the accumulation of certain lipids may be diagrammed as follows:



In fat necrosis, the fat is split into fatty acid and glycerol, the latter disappearing, the former combining with bases to form soaps. Practically always fat necrosis is produced by the action of pancreatic juice upon fat tissue, presumably through the action of the enzymes it contains. The condition may be produced experimentally by any procedure that causes escape of the pancreatic juice from its natural channels. As much as 85 per cent of the soaps formed may be insoluble. Healing follows rapidly in case of recovery; the foci may disappear as soon as eleven days after their formation. Lesions may be produced in three to five hours large enough to be visible to the naked eye, their form and size depending only on the area of the fat tissue exposed to the action of the pancreatic juice. The lesion may appear at remote points in the thoracic and pericardial cavities, or in the subcutaneous tissues, the causative agent probably being carried by the lymphatic vessels, possibly in the form of emboli of pancreatic cells.

Adipocere is the product of a process that transforms the substances of dead bodies into a wax-like material; it occurs particularly in bodies buried in very wet places or lying in water and results in an apparent replacement of the muscles and other soft parts (but not the glandular organs) by a mass consisting of a mixture of fatty acids in crystalline and amorphous form, and soap, particularly ammonium, magnesium, and calcium soaps of palmitic and stearic acids. They are formed from the original fat of the corpse.

HYDROGENATION OF OILS

The hydrogenation of oils is of tremendous commercial importance. The process is carried out at high temperatures (180° C.) and at about 10 atmospheres' pressure in the presence of finely divided nickel. The hydrogen is often bubbled in.

The lard substitutes such as "Crisco" and "Snowdrift" do not represent anywhere nearly a complete hydrogenation. If the vegetable oils were completely hydrogenated, the resulting product would be brittle and similar to stearin or tallow. "Crisco" contains 20 to 25 per

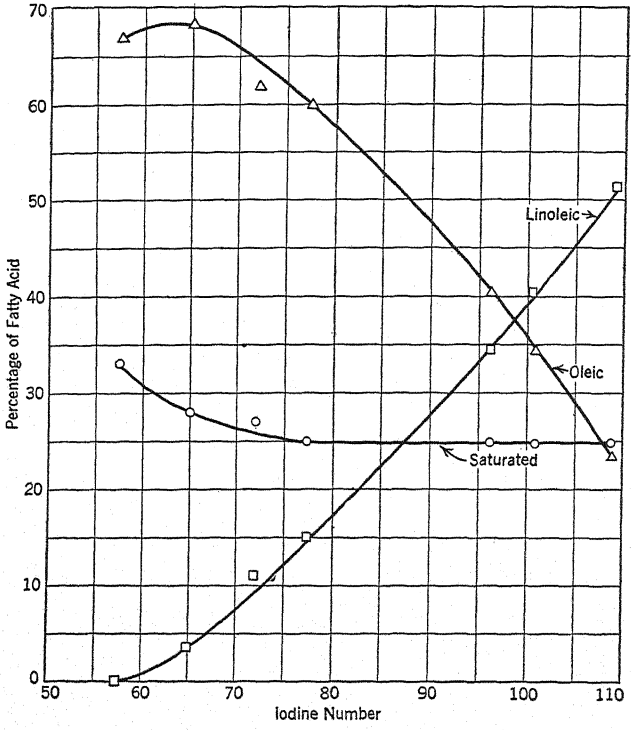


FIG. 43.—Selective hydrogenation of linoleic to oleic glycerides in cottonseed oil.

cent of saturated fat, 65 to 75 per cent oleins, and 5 to 10 per cent linoleins.

In the hydrogenation of an oil, the most unsaturated fatty acid present tends to be reduced to the next most unsaturated fatty acid, and this continues until the most unsaturated fatty acids have all been reduced to the next most unsaturated fatty acids.[64] Hilditch[65] illustrates this in Fig. 43.

Recently Hilditch[66] and co-workers have studied the process of

[64] L. Zeleny and C. H. Bailey, J. Ind. Eng. Chem. **24**, 109 (1932).
[65] T. P. Hilditch, J. Chem. Ind. **54**, 184 (1925).
[66] T. P. Hilditch and H. Paul, J. Soc. Chem. Ind. **54**, 33 T, 336 T (1935); T. P. Hilditch and W. J. Stainsby, Biochem. J. **29**, 90 (1935).

hydrogenation as an aid in determining glyceride structure. They found for example, in addition to the effect shown in Fig. 43, which illustrates the tendency of the double bond furthest away from the carboxyl to take up hydrogen, that there are additional factors which have to do with glyceride structure. The α-unsaturated acids are saturated more easily than the β-acids. They believe that the large amount of stearic acid found in pig fat arises from the saturation of the oleic acids in the α-positions, thus giving rise to a fair amount of saturated triglyceride because palmitic acid is supposed to occur in the β-position. The β-palmito-dioleins are hydrogenated before the β-stearo dioleins. If an oil is hydrogenated catalytically, the saturated glycerides are found in the same proportion as in pig fat and not as in seed fat, even if the original oil were a plant oil. There is considerably more saturated glyceride in pig fat than in seed fat for a given percentage of saturated fatty acid.

RANCIDITY

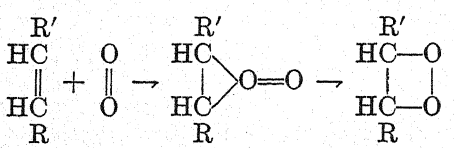
It was recognized rather early that fats undergo certain changes which cause them to acquire an unpleasant or rancid odor and taste, and a great deal of experimental work has been done to determine the factors which bring about rancidity. There is no standard definition of rancidity, but in general rancidity can be divided into three classes.

1. Oxidative rancidity, in which the fats or their constituents are oxidized by the oxygen of the air.

2. Hydrolytic rancidity, which is due to a hydrolysis of the fat with the liberation of free fatty acid. It is of importance in dairy products where there is apt to be a liberation of low-molecular-weight fatty acids such as butyric acid.

3. Ketonic rancidity, which is caused by the oxidative action of micro-organisms.

Oxidative Rancidity. Triebold[67] has given a good general review of oxidative rancidity. Engler and Weissberg[68] proposed that oxygen can and does attack the double bond in a similar manner to ozone. It forms a peroxide compound known as a moloxide which may rearrange to form a stable peroxide.



[67] H. O. Triebold, Cereal Chem. 8, 518 (1931).

[68] Engler and Weissberg, Kritische Studien über die Vorgänge der Autoxydation, p. 90, Braunschweig, 1904.

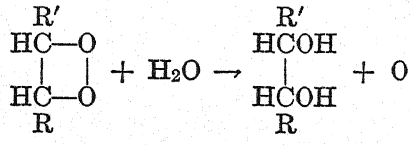
This theory of Engler and Weissberg is the forerunner of all modern theories.

The method of addition of oxygen is still a matter of dispute. Milas[69] proposed a rather complicated scheme based on the donation of unshared or exposed electrons of the auto-oxidant to the oxygen molecule with the formation of "dative" peroxides which rearrange to form the stable peroxide. Stephens[70] has criticized this theory and has pointed out that there is little evidence for it. He believes that vibrational activation is quite sufficient to account for the experimental facts.

Powick,[71] in a splendid paper, investigated all compounds that might conceivably be derived from oleic acid by oxidation and subsequent splitting. He found that only heptylic and nonylic aldehydes gave tastes and odors similar to those of rancid fat, while only epihydrin aldehyde yielded a true Kreis test although several compounds gave some color. According to Powick, oleic acid adds molecular oxygen to form oleic acid peroxide, which later loses water to form an oxide. This oxide, after addition of two molecules of oxygen, splits into heptylic aldehyde and other compounds, one of which can form epihydrin aldehyde through the loss of carbon dioxide.

The theory of Tscherick[72] differs somewhat from that of Powick by assuming that the peroxide formed is decomposed by water, giving an oxide, hydrogen peroxide, and ozone. The ozone is the active constituent.

A rather different theory was proposed by Brown.[73] He believes that a molecule of oxygen attacks the double bond, forming a fatty oxide and liberating an atom of active oxygen for every atom of oxygen taken up. The active oxygen immediately acts upon the glyceride with which it is in contact and causes it to break up into free fatty acids, aldehydes, carbon dioxide, water, and other decomposition products. Brown also suggested that, if a peroxide were formed as postulated by Engler and Weissberg, and by Tscherick, the reaction would proceed differently from those postulated by these investigators. Such a peroxide might form a dihydroxide with the liberation of active oxygen as follows:



[69] N. A. Milas, J. Phys. Chem. **33**, 1211 (1929).
[70] H. N. Stephens, J. Phys. Chem. **37**, 209 (1933).
[71] W. C. Powick, J. Agr. Research **26**, 323 (1923).
[72] A. Tscherick, Chem. Umschau **32**, 29 (1925).
[73] C. A. Brown, J. Ind. Eng. Chem. **17**, 44 (1925).

All the theories agree on the point that free oxygen attacks the unsaturated double bond with the formation of some sort of oxide. These loosely combined oxygen compounds either decompose spontaneously in water or react with water to form aldehydes, ketones, fatty acids, active oxygen, ozone, and hydrogen peroxide. No free glycerol can be detected in rancid fat, nor does the acidity of the fat need to increase with rancidity; accordingly it is believed that the reactions characteristic of rancidity may take place without hydrolysis of the triglyceride.

Development of Rancidity. The onset of rancidity is characterized by a typical series of changes. There is an induction period of a very variable length followed by the appearance of the physical signs of rancidity which in turn are accompanied by numerous chemical changes in the fat. There is a decrease in the iodine number as well as in the heat of combustion while the specific gravity and viscosity increase. After the fat is thoroughly rancid there is an increase in the saponification number as well as in the unsaponifiable matter. The amount of free fatty acid remains remarkably small, thus indicating very little hydrolysis.

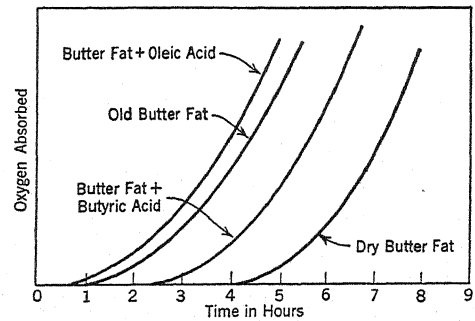


FIG. 44.—Uptake of oxygen by butter fat at 95° C. and subject to several conditions.

The products derived from the oxidation of oleic acid are mainly responsible for the intense tallowy odor of rancid fats. Oxidation of linoleic acid produces less off odor, and the oxidation of linolenic acid produces very slight off odors.

Fig. 44 shows the oxygen uptake by butter fat held at 95° C. and subject to the indicated factors.[74]

The characteristic feature is an induction period followed by a rapid absorption of oxygen. Apparently the presence of free fatty acids catalyzes the oxidation of butter fat.

The physical factors which affect rancidity are:

Moisture. Very small amounts of moisture may act as accelerators, but considerable amounts of moisture lengthen the induction period and washing of the fat is supposed to increase its keeping quality materially.

Light. Light alone cannot initiate rancidity, but it appears to have a catalytic effect on the development of rancidity. Greenbank and

[74] G. R. Greenbank and G. E. Holm, J. Ind. Eng. Chem. **16**, 598 (1924).

Holm[75] report that red and orange light is most effective in the oxidation of linseed oil and that the shorter wave lengths are ineffectual. Coe and LeClerc[76] go so far as to state that there is a strong indication that oxidative rancidity is due principally to the effect of light and is not primarily due to the formation of peroxides. They believe that the only really reliable test for rancidity is the organoleptic one, and the color tests are not effective when the fat has been protected from the light. The reaction which forms the compound or compounds responsible for the rancid taste and odor is apparently photochemical.

Fig. 45 shows the development of peroxide in samples of corn oil. The samples which had been protected from the light did not develop nearly as large peroxide values as those exposed to light, but even so the peroxide values of the protected samples at the end of one hundred days were considerably higher than the peroxide value of the exposed oils at the time rancidity developed. There was no sign of rancidity in the protected samples even after one hundred days.

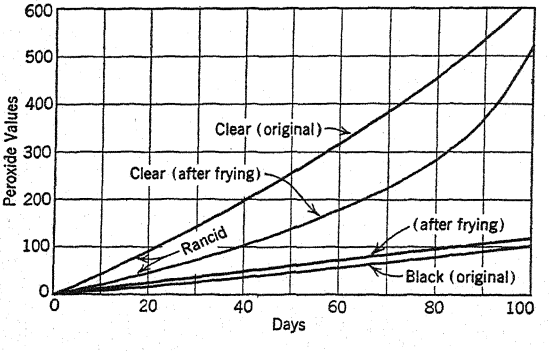


FIG. 45.—Peroxide value of corn oil before and after frying. Samples protected from light (black) and samples unprotected from light (clear).

Gases. Fats keep much better in the absence of oxygen.

Metals. Metals and their oxides may act as powerful catalysts. Thus it was found that lard stored in contact with strips of lead or copper gave a positive Kreis test in six and five days, respectively, whereas lard alone did not give a positive test until the twenty-seventh day. Metals or oxides may act as catalysts, either by virtue of the fact that they form intermediate compounds with the fat and so bring about a condition easier to oxidize, or else, according to Slansky, these substances have a directing influence upon the fat molecule and cause the unsaturated part of it to be directed toward the surface and thereby become more easily oxidizable. Recently Hamilton and Olcott[77] suggested that at least some of these pro-oxygen catalysts act by de-

[75] G. R. Greenbank and G. E. Holm, J. Ind. Eng. Chem. **25**, 167 (1933).
[76] M. R. Coe and J. A. LeClerc, J. Ind. Eng. Chem. **26**, 245 (1934).
[77] L. A. Hamilton and H. S. Olcott, Oil and Soap **13**, 127 (1936).

stroying the natural inhibitor in the fat and thus shortening the induction period.

These are various tests for oxidative rancidity, the best known being the Kreis test: to 10 cc. of the suspected oil contained in a large test tube, 10 cc. of HCl (specific gravity, 1.19) are added. The tube is closed with a rubber stopper and shaken vigorously for about thirty seconds. Ten cubic centimeters of a 0.1 per cent solution of phloroglucin in ether are then added, and the tube is closed and shaken. A red or pink color in the acid layer indicates rancidity. The intensity of the reaction is reported in terms of the highest dilution of mineral oil at which a reaction is obtainable. Unfortunately, the compound responsible for this test appears not to be the same as the one which gives the odor and taste of rancidity, and though the Kreis test usually is reliable it is not infallible. However, Kerr and Sorber[78] found that the intensity of the color of the Kreis test was approximately proportional to the degree of rancidity, and Holm and Greenbank found it to be proportional to the oxygen uptake.

There are numerous other tests for rancidity. The peroxide value[79] determined by measuring the amount of iodine liberated from potassium iodide by a thiosulfate titration is commonly used. As stated above, however, Coe and LeClerc found little correspondence between rancidity and peroxide formation if the fat is protected from light. Almost any substance which can be used as a test for peroxides can be used to test for rancidity; also, the aldehydes may be taken as a measure of rancidity and the usual tests for aldehydes used. Methylene blue reduction has been proposed by Royce.[80] Tracy, Ramsey, and Ruehe[81] have made the novel suggestion that rancidity may be detected or rather predicted by measuring the red-ox potential. They measured the potential against a saturated calomel half cell with a platinum electrode. They found, in general, that if the potential was above 0.3000, rancidity in milk was apt to develop. The test gave a rather ragged and uncertain performance. They found strangely enough that rancidity was more apt to occur in milk during the winter months than in the summer. They make the suggestion that this is due to the lack of bacteria in the winter months and thus more oxygen is available for rancidity. Yeast prevented the development of rancidity.

Schiff's reagent has been applied in testing for rancidity (C.A. **17**, 3731).

[78] R. H. Kerr and D. G. Sorber, J. Ind. Eng. Chem. **15**, 383 (1923).
[79] R. B. French, H. S. Olcott, and H. A. Mattill, J. Ind. Eng. Chem. **27**, 724 (1935).
[80] H. D. Royce, Soap **7**, 25, 38 (1931).
[81] P. H. Tracy, R. J. Ramsey, and H. A. Ruehe, Univ. Ill. Agr. Exp. Sta. Bull 389, 1933.

Ketonic Rancidity. Ketonic rancidity is brought about by the action of fungi (*Aspergillus niger* and *Penicillium glaucum*) mostly on such fats as cocoanut fat which contain some nitrogenous matter. Apparently water and a nitrogen-containing substance must be present for ketone formation to take place. It is thought that the characteristic odors produced by fungi on Roquefort cheese are not butyric acid esters but methyl ketones. Ketone rancidity gives a negative Kreis test.

Hydrolytic Rancidity. Hydrolytic rancidity is of importance in the dairy industry. It consists of the hydrolytic splitting off of fatty acid. Lipase activity plays an important rôle in rancidity of this kind.

DRYING OILS

Oils may be divided according to their degree of unsaturation into three classes: (1) non-drying oils with an iodine number below 100; (2) semi-drying oils with iodine numbers between 100 and 130; and finally (3) drying oils with an iodine number greater than 130. The principal drying oils are linseed oil from the seeds of the flax plant, tung oil or China wood oil, and soy bean oil.

These oils contain highly unsaturated fatty acids; linseed oil from the seed of the flax plant has the following approximate composition:

Fatty Acid	Per Cent
Oleic	5
Linoleic	48
Linolenic	34
Saturated	12

Tung oil is obtained from two species of *Aleurites*, a small genus belonging to the spurge family (Euphorbiaceae). Both of these species, *A. fordii* and *A. montana*, are natives of China. Tung oil has the following approximate composition:

Fatty Acid	Per Cent
Palmitic	4
Stearic	1
Elaeostearic	75
Oleic	14

The glyceride of tung oil exists in two forms: the α-form, which is the natural form, and the β- which is obtained from the α- by the action of light in the absence of air. The β-form separates as a white precipitate, and the purified glyceride melts at 61° to 62° C.

The drying oils absorb oxygen and go through a series of changes which closely resemble the process of rancidity except that a solid dried material is obtained.

It is the custom to add to the oils various substances to accelerate the drying process. These "driers" are usually metals added as lino-

leates. In the presence of a drier the induction period at the commencement of the oxygen absorption curve is absent; although the final amount of oxygen taken up is the same, the uptake is more rapid. Fig. 46, taken from the work of Rhodes and Van Wirt,[82] shows clearly the effect of a lead drier on the oxygen uptake by linseed oil. The drying of an oil seems to involve the oxidation of the ethenoid linkages in the fatty acid. The ethenoid linkages remote from the carboxyl oxidize to a peroxide, while the ethenoid linkages near the carboxyl oxidize to a non-peroxide structure and do not contribute to association as far as ordinary temperatures are concerned.

Morrell and Davis[83] have made a study of the oxidation and polymerization of α- and β-elaeostearin maleic anhydride compounds in polar and non-polar solvents. They find that the behavior of the α- and β-glycerides is very different indeed. No peroxide was formed with the α-glyceride and no polymerization took place. On the other hand, the β-glyceride underwent polymerization and peroxide formation. Its behavior in a polar solvent such as acetic acid was of a different nature from that in a non-polar solvent. Non-polar solvents were found to enhance the formation of aggregates. This is to be expected, since a non-polar solvent would tend to bring the polar groups in the glyceride together; even the saturated fatty acids form double molecules in a non-polar solvent. After the polar aggregates are formed in a polar solvent, they are transformed into an associated or polymerized micelle, with a considerable drop in peroxide value.

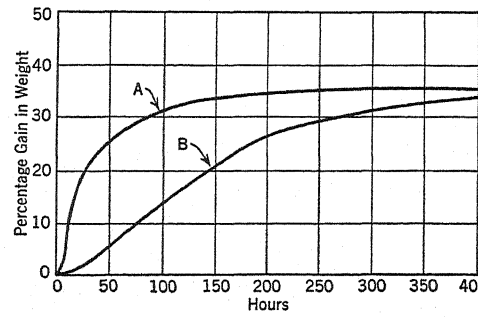
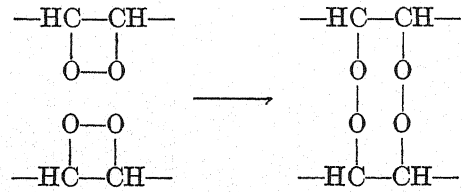


FIG. 46.—Oxygen uptake of linseed oil with and without drier. (*A*) 0.2 per cent lead as drier. (*B*) without drier.



Polar aggregates Associated or polymerized micelles

[82] F. H. Rhodes and A. E. Van Wirt, J. Ind. Eng. Chem. **15**, 1135 (1923).
[83] R. S. Morrell and W. R. Davis, Trans. Faraday Soc. **32**, 209 (1936).

Accompanying the drop in the peroxide value is an increase in viscosity, as is shown in Fig. 47.

The behavior of the glyceride in a non-polar solvent is quite different. Here the peroxide value remains constant, which indicates that the linkages between the glyceride molecules, in these two cases, are very different.

Oxidation is not always necessary for the polymerization of a drying oil, at any rate for the formation of a gel which is probably a polymer. For example, tung oil, if heated to about 280° C. in the absence of oxygen, will form a stiff gel.

Polymerization in drying oils can be demonstrated either from molecular-weight determination or from viscosity studies similar to those

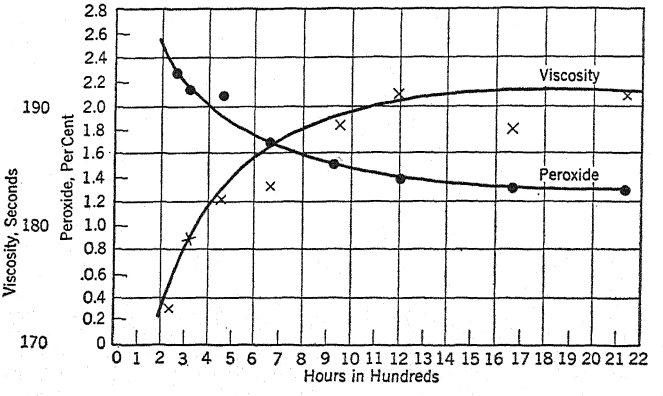


FIG. 47.—Viscosity and peroxide changes of β-elaeostearin maleic anhydride in a polar solvent.

of Staudinger. As Staudinger has pointed out, an increase in the viscosity of an oil dissolved in some solvent, upon oxidation, must mean that chains of molecules are forming and that we are not dealing with spheres, because, with spherical particles, the viscosity of a solution is independent of a particle size.

AUTO-OXIDATION

The term auto-oxidation was originally applied to all oxidation reactions taking place spontaneously at room temperature. Later it came to include such reactions at any temperature, and today, the term has apparently lost most of its meaning. Perhaps it would be better to use the expression "oxidation reactions."

Elaborate theories[84] have been built up to explain the effect which certain substances have on such reactions. For example, it has been found that small amounts of hydroquinone will greatly diminish the speed of oxidation of benzaldehyde. Such substances are called antioxygens or antioxidants or inhibitors. They are found to play a rôle in a variety of reactions. For example, tetraethyl lead, or a decomposition product formed therefrom, slows down the burning of gasoline and so prevents the "knocking" in a motor. At the present time, the idea is favored that a chain mechanism is involved in the drying of oils as well as in rancidity, and that this is generally true of all such reactions.[85] The effect of an inhibitor is to break the chain and so decrease the speed of reaction. The drying of oils or the process of fat becoming rancid follows the pattern of chain reactions. Certain "hot" or activated molecules are produced, and these collide with other molecules which are in turn activated and are thus able to become oxidized and activate molecules, and so on. If an inhibitor is present, the activated oil molecule is apt to collide with it and so lose its energy, and thus the chain is broken. The inhibitor is at the same time destroyed, and is finally used up, and the oxidation of the fat then proceeds normally. Thus, the inhibitor is responsible for the induction period. Polymerization is also thought to be a chain reaction. In fact, the two reactions are probably closely connected. The activation energy received from oxidation may serve to activate molecules for the polymerization reaction. Chain reactions may be many thousands of reacting molecules long.

Olcott[86] has conducted a study of the antioxidants for the oxidation of lard, with the idea of determining the relation of the structure of the molecules to their antioxidant properties. He found that apparently a free phenolic group is necessary, and furthermore, the position of this group in relation to other groupings in the molecule is important. For example, resorcinol was inactive while catachol and hydroquinone were active. If the phenolic group is combined with another group, the antioxidant powers are largely lost. There is no simple relation between reducing power of the molecule and its antioxidant properties. Olcott defined the antioxidant power of a compound in terms of its antioxidant index, which is the ratio of the length of the induction period with the antioxidant present to that in the absence of the antioxidant. Table XXI shows the antioxidant indices of a number of compounds in lard at 75° C.

Commercial lecithin has been used as an antioxidant. The advantage

[84] C. Moreau and C. Dufraisse, Chem. Rev. **3**, 113 (1926).
[85] J. A. Christiansen, Trans. Faraday Soc. **24**, 714 (1928).
[86] H. S. Olcott, J.A.C. S. **56**, 2492 (1934).

TABLE XXI

The following results were obtained using 0.5 mg. of substance to 5 grams of lard.

Inactive Compounds

Hydroquinone diacetate	Anthraquinol
Hydroquinone monobenzoate	1,5-Dihydroxy anthraquinol
Hydroquinone dibenzoate	2,4,6-Tribromo resorcinol
Hydroquinone di-(*p*-nitro-benzoate)	Saligenin
p-Dimethoxybenzene	1,4-Cyclohexanediol
Hydroxydroquinone triacetate	Thymoquinone
Apional tetracetate	Triquinoyl
Hexahydroxybenzene	Cyclohexanol
Dipyrogallol tricarbonate	Tannic acid
1,4- Naphthalenediol	Tartaric acid
Citric acid	Maleic acid

Antioxidants and Indices

Catechol	41	Pyrogallol carbonate	2
Hydroquinone	38	Apionol	20
Hydroquinone monomethyl ether	6	Naphthoresorcin	5
Toluhydroquinone	7	1,8-Naphthalenediol	20
Thymohydroquinone	2	α-Naphthol	22
Hydroxyhydroquinone	60	Quinone	4
Pyrogallol	60+	Toluquinone	2
		β-Naphthoquinone	8

Pro-oxidants

Carotene	0.5	Xanthophyll	0.7
Lycopene	0.5	Perbenzoic acid	0.5

of lecithin as an antioxidant is that it is a natural product and can be used in foods. Olcott and Mattill[87] report, however, that commercial lecithin has only moderate antioxygenic action on refined cottonseed oil, little effect on lard, and none at all on mixtures of lard and cod-liver oil. They point out that commercial lecithin contains only very small amounts of true lecithin, and that the antioxygen properties of commercial lecithin are due to the presence of cephalin. Purified lecithin was found to have no antioxygen properties at all. They suggest that the portion of the cephalin molecule which is responsible for the antioxygen character is probably the monobasic phosphoric acid radical.

Olcott and Mattill *et al.*[88] have made a rather thorough study of some of the natural antioxidants from the unsaponifiable fraction of a number of oils. They term such substances "inhibitols." They have

[87] H. S. Olcott and H. A. Mattill, Oil and Soap **13**, 98 (1936).

[88] H. A. Mattill, J.B.C. **90**, 141 (1931); H. S. Olcott, J.B.C. **107**, 471 (1934), **110**, 695 (1935); H. S. Olcott and H. A. Mattill, J.B.C. **93**, 59, 65 (1931); R. B. French, H. S. Olcott, and H. A. Mattill, J. Ind. Eng. Chem. **27**, 724 (1935); H. S. Olcott and H. A. Mattill, J.A.C. S. **58**, 1627 (1936).

been able to obtain only the inhibitol from lettuce in a crystalline state, although inhibitols are present in tomatoes, carrots, alfalfa, spinach; in wheat germ, cottonseed, corn, sesame, palm, soy bean, and peanut oils; and, as they state, probably in many other vegetable substances. No demonstrable amounts of inhibitols are present in yeast or lard, or in olive (trace), cod-liver, palm kernel, or castor oils. Inhibitols from various sources are distinctly different in their properties. The inhibitols from wheat germ and cottonseed oils very closely resemble vitamin E in their physical and chemical properties. In fact, the resemblance is so marked that the method used for obtaining the most active concentrates from these two oils is exactly the same as that described for obtaining vitamin E concentrates, and, indeed, so far it has not been possible to separate the vitamin from these two inhibitols. The inhibitols from lettuce and tomatoes can be separated, however, and it was thus possible to show that vitamin E is not an antioxidant.

The inhibitol concentrates from wheat germ, cottonseed, and palm oils are light yellow transparent oils of medium viscosity, which do not crystallize on long standing. Under ordinary laboratory conditions they are stable for years. They are soluble in fat solvents. They possess at least one OH group and appear to contain a difficultly hydrogenated double bond which is essential to their activity. Inhibitols are always inactivated by reagents which combine with a free hydroxyl group.

The inhibitols protect lard, purified fatty acids, and esters. They are, ineffective, however, as antioxidants for the vegetable fats and oils from which they are prepared.

Olcott and Mattill[89] have recently reported a preliminary classification of inhibitors. They found that the crude ethyl esters of hydrogenated cottonseed oil and other vegetable oils, prepared by ethanolysis with absolute alcohol and dry HCl gas, are protected to a remarkable degree by oxalic, malonic, maleic, citric, and other aliphatic dibasic acids, also by phosphoric and sulfuric acids and cephalin. Hydroquinone and inhibitol concentrates are much less effective antioxidants. When the esters are purified by distillation, they are no longer protected by these acids but are protected by inhibitols and hydroquinone. The acid inhibitors and inhibitol concentrates demonstrate a remarkable synergistic effect when used together in the distilled ester preparations, purified fatty acids and esters, and lard, but not in vegetable fats or their crude esters. On the basis of these observations, inhibitors are tentatively classified in three groups: (*a*) acid inhibitors, (*b*) inhibitols and hydroquinone, (*c*) phenolic inhibitors.

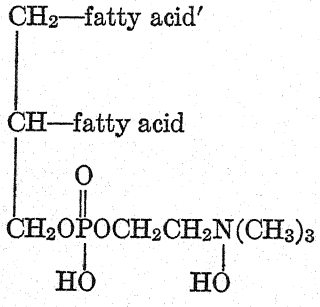
[89] H. S. Olcott and H. A. Mattill, September, 1936, meeting of the American Chemical Society at Pittsburgh, Pa.

CHAPTER VI

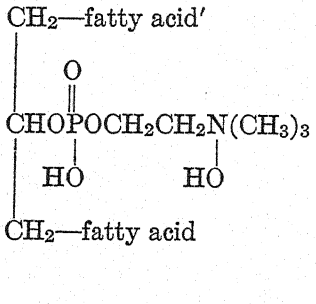
THE PHOSPHOLIPIDS

The early work on the phospholipids was done by J. L. W. Thudichum, who was born on August 27, 1829, in Büchingen in Hessen and died on September 7, 1901, in London. He published largely in the British Government Reports, and his work remains classical in the field. The best recent references of a general nature are "Lecithin and Allied Substances" by Maclean and Maclean, and "Die Chemie der Cerebroside und Phosphatide" by Thierfelder and Klenk.

There are three types of phospholipids: lecithin, cephalin, and sphingomyelin. The first two have nitrogen to phosphorus ratios of 1 : 1; sphingomyelin has the ratio 2:1. There have been numerous reports of other phospholipids. These have always turned out to be either decomposition products of the above lipids or a mixture of the above. Some workers have even doubted the existence of the phospholipids. For example, Barbieri, after repeated attempts, completely failed to obtain any trace of lecithin from three thousand eggs! (Cited by Leathes and Roper, "Fats.")



α-Lecithin



β-Lecithin

Rae[1] reports egg lecithin to occur predominately in the β-form; liver lecithin contains about equal amounts of the α- and β-forms; whereas brain lecithin, brain cephalin, and calcium phosphatidate occur mainly in the α-form.

[1] J. J. Rae, Biochem. J. 28, 152 (1934).

Preparation. It is neither necessary nor desirable to enter into the host of details connected with the preparation of lecithin. For these the student should consult the references. With any of the methods it is common practice to dry the tissue, and if the tissue is an organ such as a heart or liver to grind it. Various drying agents, as well as vacuum ovens, have been used. Acetone is very useful as a drying agent.

After the tissue has been dried it is necessary to extract with some solvent. Either alcohol or ether is commonly used. Frequently both are employed since it seems that it is not possible to remove all the lecithin with an ether extraction. The dried tissue is shaken with warm alcohol and then continuously extracted with ether for several hours.

At this point the usual sequence is to employ the method of Levene and Rolf,[2] which depends upon the separation of the cadmium chloride salt of lecithin, the liberation of this salt with ammonia dissolved in methyl alcohol, and final emulsification of the lecithin in acetic acid solution. This last step seems to the author to be an extremely drastic treatment for such a labile substance as lecithin. Maltaner[3] has suggested a modification of Levene's method omitting the acetic acid treatment. Suzuki and Yokayama[4] have proposed a method for separating the α-form from β-lecithin through the cadmium chloride salt.

The preparation and purification of lecithin are not simple. There is danger of contamination with the cerebrosides, sphingomyelin, and especially with cephalin. Most serious of all, however, is the possibility of decomposition of the lecithin. The highly unsaturated fatty acids of lecithin oxidize with amazing rapidity, and the exposure to air will change a pure sample of lecithin which is perfectly clear and wax-like to a yellow or brown in a few minutes through oxidation. The fatty acids are also hydrolyzed off very easily. Lecithin, to be considered a pure undenatured product, should have an isoelectric point of 6.7 or higher, contain no amino nitrogen, and yield a phosphorus to nitrogen ratio of one.

Properties. Lecithin, as indicated, is a substance with a clear paraffin-like appearance which quickly turns yellow on exposure to air. It softens to more or less of an oil at 60° C. It is to be expected that the softening point of lecithin would depend greatly upon the contained fatty acids and also upon the proportion of the α- and β-isomers.

Lecithin forms double salts with mercuric chloride, cadmium chloride,

[2] P. A. Levene and I. Rolf, J.B.C. **72**, 587 (1927).
[3] F. Maltaner, J.A.C. S. **52**, 1718 (1930).
[4] B. Suzuki and Yokayama, Proc. Imp. Acad. Tokyo **6**, 341 (1930).

and platinic chloride. It adds bromine and iodine. The calculated iodine number for palmityl oleyl lecithin is 32.6; for palmityl arachidonyl lecithin, 126.95; for stearyl oleyl lecithin, 31.5; and for stearyl arachidonyl lecithin, 122.6. Oxidation, of course, reduces these values greatly. The iodine number of the fatty acids of lecithin from animal tissue is seldom below 100 if proper precautions have been exercised in the determination.

Lecithin may be hydrogenated in an alcoholic solution with hydrogen in the presence of colloidal platinum. The resulting product is called hydrolecithin; it is a white crystalline non-hydroscopic powder, insoluble in alcohol and acetone, and only slightly soluble in ether.

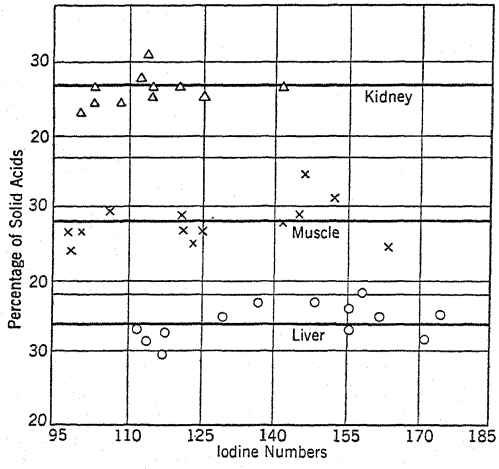


FIG. 48.—Showing constancy of percentage of solid acids in mixed phospholipid fatty acids of widely different degrees of unsaturation.

Natural lecithin is optically active, and an ethyl alcohol solution rotates the plane of polarized light to the right. The optical activity of natural lecithin is no doubt due to the α-form. The β-form should be optically inactive.

Fatty Acids of Lecithin. Lecithin is easily hydrolyzed into various fragments. The fatty acids appear to split off first. There are statements in the literature to the effect that each lecithin molecule contains one saturated and one unsaturated fatty acid. Apparently this is wholly untrue as the recent work of Sinclair shows. Sinclair[5] studied the fatty acid of the phospholipid from kidney, muscle, and liver of rats and in dog's blood by means of the Twitchell lead salt method and separated the liquid (unsaturated) from the solid (saturated) fatty acid. He was able to show that the liver phospholipids contain 33 per cent, the muscle 27 per cent, and the kidney 26 per cent saturated fatty acid, the rest of the fatty acids showing varying degrees of unsaturation. The ratio between the saturated and unsaturated fatty acids in the phospholipid is remarkably constant. Fig. 48 shows Sinclair's results.

In an extensive study of the composition of the component fatty

[5] R. G. Sinclair, J.B.C. 111, 261 (1935).

acids of the beef liver phospholipids by Snider and Bloor[6] it was found that the ratio of unsaturated to saturated acids was about 55 : 40. The unsaturated acids contained representatives of the C_{18} and C_{20} series, the highly unsaturated members predominating. The amounts were as follows: 21 per cent oleic, 45 per cent linoleic, and 31 per cent arachidonic acid. The saturated acids were composed of 17 per cent stearic and 29 per cent palmitic acid.

In an attempt to gain more information concerning the nature of the unsaturated fatty acids in the blood in eczema, as well as in other pathological conditions, Hansen and Hessler[7] have devised a method for the isolation of the fatty acids present in the phospholipid fraction. Although extensive studies have not been made, preliminary findings indicate that the phospholipid fatty acids of the serum tend to have a higher average molecular weight and lower iodine value than the total fatty acids. This finding rather conflicts with the evidence based on the indirect determination of the phospholipid fatty acids by the oxidative methods. The iodine numbers, however, agree closely with the findings of Bloor, wherein large samples of blood from various animals were used. The acetone-soluble fraction of the serum fatty acids (neutral fat plus cholesterol esters) was found to contain slightly shorter chains and somewhat more unsaturated acids than the phospholipid (acetone-insoluble) fraction. The phospholipid fatty acids from the serum of eczematous infants seem to be less unsaturated than those from normal infants.

Levene and Rolf[8] report stearic, palmitic, oleic, linoleic, and linolenic acids in soy bean lecithin.

Choline. Shaking an aqueous emulsion of lecithin with sulfuric acid splits off choline. Alcoholic hydrocholoric acid also hydrolyzes off the choline. Choline is a very strong base; in fact, it is said to be as strong a base as sodium hydroxide. It has some physiological action: it is a depressant, causing a fall in blood pressure. When putrefying bacteria act upon choline, the alcoholic side chain suffers dehydration, forming neurine, $(CH_3)_3NOHCH{=}CH_2$. Neurine is very poisonous. Further decomposition results in the trimethyl amine, betaine

$$\begin{array}{l} CH_2\text{—}CO \\ \quad\quad\quad | \quad\quad | \\ (CH_3)_3N\text{——}O \end{array}$$

The suggestion has been made that the plant lecithins have betaine as a base in place of choline. Choline forms an insoluble salt with platinum

[6] R. H. Snider and W. R. Bloor, J.B.C. **99**, 555 (1933).
[7] A. E. Hansen and Lyle Hessler, private communication.
[8] P. A. Levene and I. P. Rolf, J.B.C. **62**, 759 (1924).

chloride and also an insoluble iodide. Both compounds have been used in the quantitative estimation of choline.

Glycerophosphoric Acid. It is very difficult to hydrolyze the glycerophosphoric acid, which results from splitting the fatty acids and choline from lecithin, into glycerol and phosphoric acid. It is completely resistant to the action of bases and is hydrolyzed by dilute acids only after prolonged boiling, and then only slowly. It is best split by an enzyme which is found in yeast and in many animal and plant sources. Glycerophosphoric acid is a relatively strong dibasic acid. The third hydrogen of the phosphoric acid is tied to the glycerol, and the pK_a values of glycerophosphoric acid are 1.4 and 6.32. The second hydrogen of glycerophosphoric acid, in lecithin, is bound through an ester linkage to the choline, thus leaving the primary hydrogen of the phosphoric acid free. This means that lecithin is really a very strong acid. The strong hydroxyl group of choline is also free, however, which gives to lecithin an ampholytic nature with a true isoelectric point.

Synthesis. Grün and Limpächer[9] claim to have synthesized lecithin according to the following set of reactions:

$$\begin{array}{l}\text{CH}_2\text{—fatty acid}\\ |\\ \text{CH—fatty acid} + \text{P}_2\text{O}_5\\ |\\ \text{CH}_2\text{OH}\end{array} \qquad \begin{array}{l}\text{CH}_2\text{—fatty acid}\\ |\\ \text{CH—fatty acid}\\ |\\ \text{CH}_2\text{OPO}_2\end{array}$$

At this point choline bicarbonate is added which yields

$$\begin{array}{l}\text{CH}_2\text{—fatty acid}\\ |\\ \text{CH—fatty acid} + \text{HOC}_2\text{H}_4\text{N(CH}_3)_3\text{X}\\ |\\ \text{CH}_2\text{OPO}_2\end{array} \qquad \begin{array}{l}\text{CH}_2\text{—fatty acid}\\ |\\ \text{CH—fatty acid}\\ |\\ \quad\text{O}\\ \quad\|\\ \text{CH}_2\text{OPOCH}_2\text{CH}_2\text{N(CH}_3)_3\\ \quad|\qquad\qquad|\\ \quad\text{HO}\qquad\quad\text{X}\end{array}$$

If this compound be treated with dilute acid, distearyl lecithin is obtained. The choline is used as the bicarbonate in order to mask the strongly basic hydroxyl group on the nitrogen; otherwise, this would be the group which would react with the phosphoric acid residue, and a salt would be formed and not the ester. Grün makes much of the ease with which choline is hydrolyzed off and claims to be able to determine the amount of salt formed by means of a basic titration using phenolphthalein as the indicator. The synthetic lecithin had the appearance and

[9] A. Grün and R. Limpächer, Ber. **59**, 1350 (1926); **60**, 147 (1927).

properties of natural lecithin. Both α- and β-lecithin were synthesized, but since they had identical properties it seems probable that there had been a migration of the phosphoric acid and that only one form was obtained.

Kabashima and Suzuki[10] also reported the synthesis of lecithin. The silver salt of dipalmityl-β-glycerophosphoric acid reacted with bromocholine picrate and dipalmityl-β-lecithin resulted. The synthetic product had the properties of the natural product.

Acid-base Dissociation. Fischgold and Chain[11] titrated the phospholipids in a mixture containing 19 volumes benzene and 1 volume absolute ethyl alcohol which yielded a solvent having a dielectric constant of about 3.5. In such a medium it is possible to titrate quantitatively the phosphoric acid group whereas in alcohol or water this is not possible. It is also possible to titrate the amino group in cephalin quantitatively. On the other hand, it is not possible for the quaternary ammonium cation $N(CH_3)_3^+$ to show an acid character and to give up H^+-ions. They come to the conclusion that, in an aqueous medium, lecithin exists entirely as a zwitter ion and that cephalin does also to a very large extent. In other solvents, *e.g.*, those with a lower dielectric constant, lecithin still exists as a zwitter ion while cephalin may ionize according to the classical view. Sphingomyelin behaves very much like lecithin. Numerous titration curves have been run on lecithin. Jukes[12] titrated lecithin and cephalin in 98 per cent ethyl alcohol. Cephalin was found to bind alkali and to have a pK_a value of 8.9, but lecithin did not bind alkali. On the other hand, both phospholipids bound acid at higher hydrogen-ion concentrations and both had pK_b values of about 1.1. He suggests, as is no doubt true, that lecithin exists as a zwitter ion and its reactions with hydrogen ions would be

$$\mathrm{RO{-}\underset{\underset{\mathrm{O}}{\|}}{\overset{\overset{\mathrm{O^-}}{|}}{P}}{-}OCH_2CH_2\overset{+}{N}(CH_3)_3} + H^+ \rightleftarrows \mathrm{RO{-}\underset{\underset{\mathrm{O}}{\|}}{\overset{\overset{\mathrm{HO}}{|}}{P}}{-}OCH_2CH_2\overset{+}{N}(CH_3)_3}$$

Fabish[13] investigated the buffer capacity of lecithin and cephalin. Fig. 49 shows some of his results. These results indicate that the buffering capacity of lecithin is very small. Cephalin apparently had a somewhat larger capacity. Fig. 50 shows the data obtained by Fischgold and Chain[14] upon titrating fresh lecithin in 90 per cent alcohol (400

[10] I. Kabashima and B. Suzuki, Proc. Imp. Acad. Tokyo **8**, 492 (1932).
[11] H. Fischgold and E. Chain, Proc. Roy. Soc. **B117**, 239 (1935).
[12] T. H. Jukes, J.B.C. **107**, 783 (1934).
[13] W. Fabish, Biochem. Z. **242**, 121 (1931).
[14] H. Fischgold and E. Chain, Biochem. J. **28**, 2044 (1934).

mg. in 30 cc.). Curve III shows the presence of an acid with a pK_a of about 7.1. A separate titration of stearic acid in 90 per cent alcohol

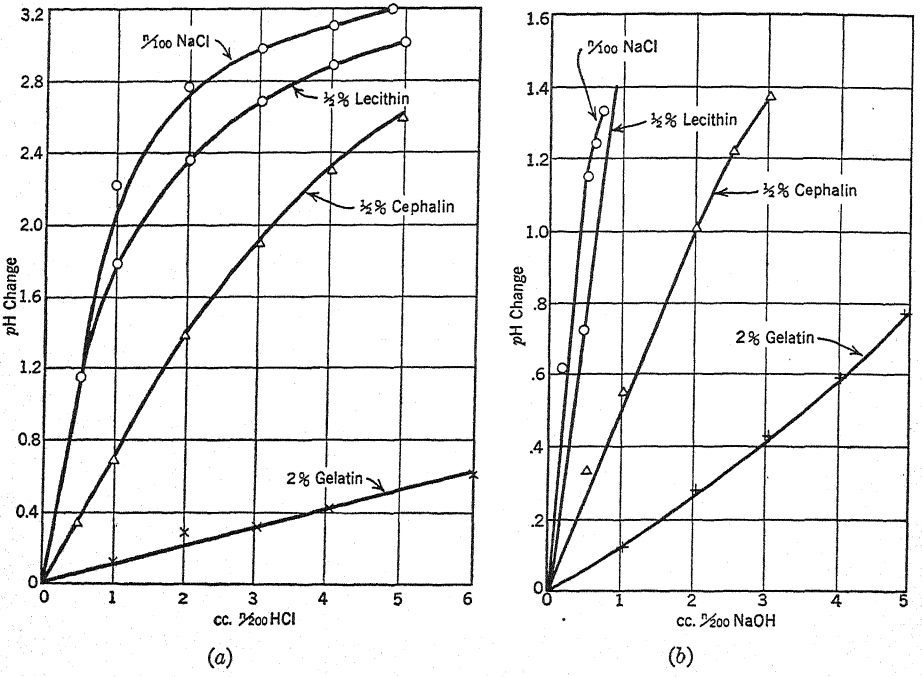


FIG. 49.—Buffering power of lecithin and cephalin.

yielded a pK_a of 7.1, indicating that the inflection in Curve III is due to a fatty acid being split from lecithin.

It is possible to find in the literature a variety of values for the isoelectric point of lecithin as shown in Table XXII.

TABLE XXII

ISOELECTRIC POINTS REPORTED FOR LECITHIN

Price and Lewis (1933)	2.7
Chain and Kemp (1934)	6.7
Fujii (1924)	2.7
Sueyoshi and Kawai (1932)	4.7
Rona and Deutsch (1926)	1.75
Remesow (1930)	2.0–2.8
Bull and Frampton (1936)	6.7
Brandenburg de Jong	2.7

The older values for the isoelectric point were all around 2.7. Apparently, this low value is due mainly to the oxidation and hydrolysis of the fatty acids, as Chain and Kemp[15] point out in their very careful paper. They obtained experimentally an isoelectric point of 6.7, but they argue, from theoretical considerations, that it should be closer to

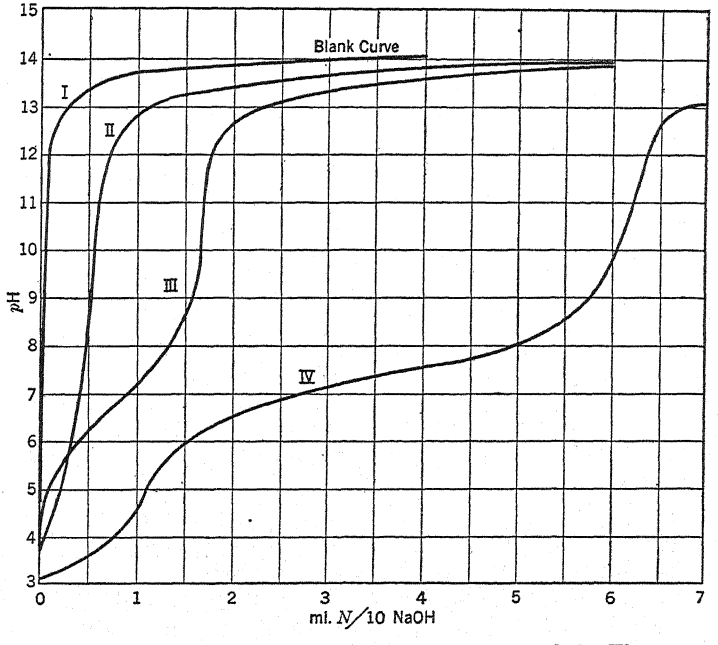


FIG. 50.—Titration curves of lecithin in 90 per cent ethyl alcohol. The curves have the following meaning: I blank, II fresh lecithin, III back titration immediately after II, IV back titration after 66 hours.

7.5 and that the low values are due to hydrolysis of small amounts of fatty acid.

Sueyoshi and Kawai[16] determined the isoelectric point of lecithin prepared according to the method of Sueyoshi and found it to be 4.7. The reader will recall that the method of Sueyoshi[17] depends, for the isolation of lecithin, on its solubility in alcohol as contrasted with the relative insolubility of cephalin. This method employs low temperatures and appealed to Bull and Frampton[18] as being a gentle and safe one for

[15] E. Chain and I. Kemp, Biochem. J. **28**, 2052 (1934).
[16] Y. Sueyoshi and K. Kawai, J. Biochem. Japan **15**, 277 (1932).
[17] Y. Sueyoshi, J. Biochem. Japan **13**, 145 (1931).
[18] H. B. Bull and V. L. Frampton, J.A.C. S. **58**, 594 (1936).

preparing lecithin. These authors found, however, that cephalin is fairly soluble in alcohol in the presence of lecithin and that this procedure yielded a mixture of lecithin and cephalin which had a fairly constant composition and an isoelectric point close to *p*H 4.0. This, then, was the substance with which Sueyoshi and Kawai were dealing. Bull and Frampton were able to fractionate this mixture to some extent with hot acetone. As the acetone cooled, fractions separated which had varying amino nitrogen (cephalin) content. When these amino nitrogen values were plotted against the isoelectric point of the fractions the graph shown in Fig. 51 was obtained. Evidently the presence of cephalin greatly lowers the isoelectric point of lecithin, which is to be expected since cephalin is a much more acid substance than lecithin, having the strong acid group from phosphoric acid and a weak amino group. This wide difference in isoelectric point suggests the possibility of coacervate formation between lecithin and cephalin over a considerable range of *p*H.

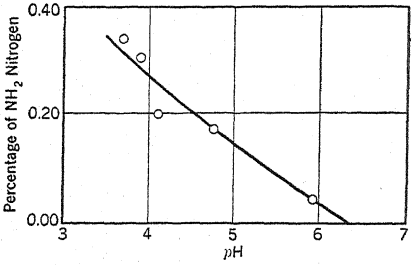


FIG. 51.—Effect of small amount of cephalin on the isoelectric point of lecithin.

Price[19] studied the change of the isoelectric point of lecithin which had been stored under nitrogen. He found the initial high value to fall progressively with time until a value of 2.7 was reached. He agrees with Chain and Kemp that this fall in isoelectric point is due to the splitting off of the fatty acids but maintains that aged lecithin is more nearly like natural lecithin than fresh lecithin is. This last point needs a great deal of consideration before it can be accepted.

No doubt there is considerable difference between fresh and aged lecithin, not only in regard to their electrokinetic behavior, but also their lyophilic qualities. Fresh lecithin is much more hydrophobic than aged lecithin. The author regards it as unfortunate that so many of the studies on the physicochemical properties have been conducted on aged lecithin and not on fresh, pure samples.

LECITHINASES

Some confusion exists in regard to the lecithinases and, as pointed out by Contardi and Ercoli,[20] there are really four kinds, corresponding to the four ester linkages in lecithin:

[19] C. W. Price, Biochem. J. 29, 1021 (1935).
[20] A. Contardi and A. Ercoli, Biochem. Z. 261, 275 (1933).

1. Lecithinase A is capable of liberating only one molecule of the fatty acid. It was early shown that cobra venom contained an enzyme which was capable of splitting off the unsaturated acids in lecithin, with the production of powerfully hemolytic lysolecithin. King and Dolan[21] were able to use rattlesnake venom as a source of their enzyme. These authors investigated the production of soluble phosphorus from lysolecithin by the action of various enzymes. The enzyme from the mucosa of the small intestine hydrolyzed lysolecithin at a much greater rate than it attacked lecithin; also bone phosphatase, which has very little action on lecithin, hydrolyzed lysolecithin at an appreciable rate.

2. Lecithinase B splits both fatty acids away from the lecithin molecule. This enzyme is found in rice hulls. It is well known that an extract of rice hulls is capable of preventing and curing beriberi. The suggestion has been made by Contardi and Ercoli that a relation exists between these phenomena.

3. Lecithinase C can only hydrolyze off the choline.

4. Lecithinase D splits the phosphoric acid away from the glycerol molecule, *i.e.*, it is a true glycerophosphatase.

Only lecithinases A and B have been identified as having separate existence.

King[22] investigated the production of soluble phosphorus from pure lecithin by the action of lecithinases from various tissues. These enzymes were probably mixtures from several of the four types of lecithinases. He found a *p*H of 7.5 to be optimal. Lecithinases have wide occurrence. The relative lecithinase activity of different tissues is as follows (in decreasing order): kidney, small intestine, spleen, liver, testes, pancreas, large intestine, brain, ovaries, bone, suprarenals, lung, blood vessels, cardiac muscle, skeletal muscle. King[23] has extended his study of the lecithinases. He measures the extent of enzyme action as before by following the soluble phosphorus. Lecithinases appear to act most rapidly at body temperature, as shown in Fig. 52. The effect of substrate concentration on lecithinase hydrolysis is shown in Fig. 53. Hydrolecithin is attacked as fast as its parent lecithin; cephalin and phosphatidic acid are attacked somewhat more slowly, the latter without showing any marked dependence on *p*H. Synthetic lecithin (Grün) and distearyl phosphate are attacked by the enzyme at a lower rate and without showing any *p*H optima. The rates of hydrolysis of natural and synthetic lecithin are compared in Fig. 54.

The rates are indeed so different that there is doubt that synthetic

[21] E. J. King and M. Dolan, Biochem. J. **27**, 403 (1933).
[22] E. J. King, Biochem. J. **25**, 799 (1931).
[23] E. J. King, Biochem. J. **28**, 476 (1934).

lecithin really has the constitution of natural lecithin. Lysolecithin is hydrolyzed about twice as fast as natural lecithin, and brominated lecithin is hydrolyzed at a very much greater rate than natural lecithin.

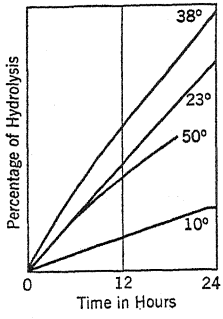


FIG. 52.—Effect of temperature on lecithinase activity.

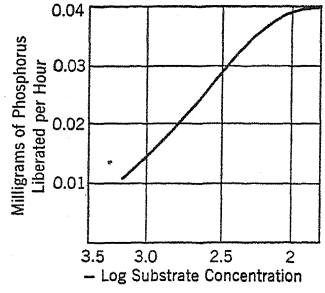


FIG. 53.—Effect of substrate concentration on lecithinase hydrolysis.

Surface Activity. Lecithin has always been regarded as a very capillary-active substance. This statement should be qualified, however. In the first place, the capillary activity of any ampholyte is strongly dependent upon the hydrogen-ion concentration, and lecithin

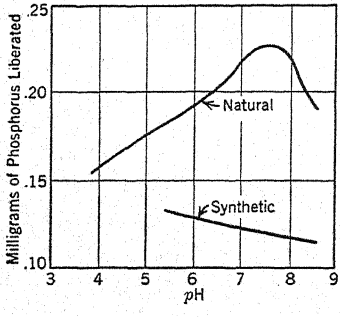


FIG. 54.—Rate of hydrolysis of natural and synthetic lecithin as a function of *p*H.

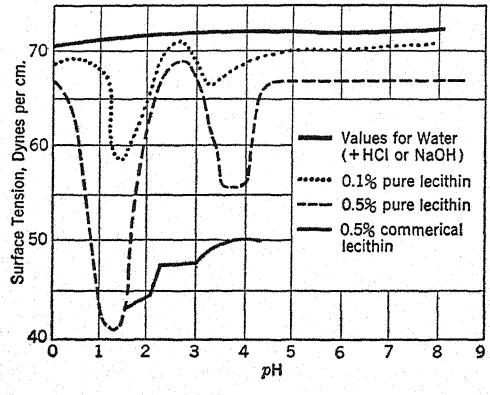


FIG. 55.—Capillary activity of lecithin (partly decomposed) as a function of pH.

is no exception. Fig. 55 shows the measurements of the surface tension of aqueous suspensions of lecithin.[24] Although this lecithin had an isoelectric point of 2.7 and therefore must be regarded as partly hydro-

[24] H. I. Price and W. C. Lewis, Biochem. J. **23**, 1031 (1929).

lyzed, the data demonstrate what might be expected from pure lecithin.

The author has not conducted quantitative studies, but pure lecithin as obtained in a careful preparation is not extremely capillary-active. It is only when lecithin is combined either chemically or by an adsorption complex to some other substance that it acquires its remarkable capillary activity. For example, commercial lecithin, among other things, always contains about 25 per cent carbohydrate, as Horvath[25] has pointed out. And to this combination commercial lecithin owes its emulsifying power. Likewise, Sell, Olsen, and Kremers[26] found lecithin in combination with protein to be very much superior as an emulsifier to pure lecithin.

Leathes[27] has reported some interesting physical studies on lecithin. He notes that if lecithin is observed under the microscope in contact with water it can be seen that growths are proceeding out from the lecithin. These are the myelin forms. It is found that calcium ions behave very differently from the other ions investigated, apparently inhibiting myelin formation or at any rate modifying it in the presence of other ions. In the presence of cholesterol myelin forms are not inhibited by the Ca^{++}, and in general cholesterol promotes the growths in the presence of other ions.

Leathes also studied the behavior of some of the cell lipids in thin films on water. He found the area occupied by a fatty acid chain, whether as a fatty acid, in its ethyl ester, or in its triglyceride, to be 21 sq. Å. in the condensed film, which agreed with Adam's previous findings. In an expanded film of palmitic acid under standard temperature and compression this area was 42 sq. Å. At room temperature and standard pressure the area occupied by each fatty acid chain in lecithin was found to be 56.6 sq. Å., and at 4.5° C. it was 52.6 sq. Å. The curve of diminishing area with increasing pressure up to 60 atmospheres and at low temperature showed no signs of the film condensing. The figures seem to show that the glycerylcholylphosphoric acid, which links the two fatty acid chains in lecithin, occupies much greater space than the two carboxyl groups which it displaces in the fatty acid, and thereby separates the carbon chains and so reduces the potential cohesive force which these chains exert upon one another. Hydrolecithin was found to give a condensed film, under standard conditions, each carbon chain displacing 29 sq. Å., and even under the high compression of 200 atmospheres the area was barely as small as that for the same chain in a fatty-acid molecule under a fortieth of this compression. The large

[25] A. A. Horvath, Ind. Eng. Chem. News Edition, **13**, 89 (1935).
[26] H. M. Sell, A. G. Olsen, and R. E. Kremers, Ind. Eng. Chem. **27**, 1222 (1935).
[27] J. B. Leathes, Lancet **208**, 803, 853, 957, 1019 (1925).

polar group in lecithin thus definitely prevents close packing of the fatty acid chains, reduces their cohesive force, and eases the passage of foreign molecules between them. Cholesterol, if it occurs as a monomolecular film under standard conditions, is 38.3 sq. Å. per molecule; 170 atmospheres reduced this area but 3 per cent. The effect of cholesterol on the behavior of lecithin and palmitic acid, after deducting for the amount of cholesterol and assuming that the effect is one-sided, is shown in the graphs in Fig. 56. By comparing with the untreated substances it is seen that cholesterol has tended to reduce the area occupied per molecule, the effect being more noticeable with expanded films and more striking for fatty acids than for lecithin, which suggests that it is a function of the cholesterol with the carbon chains rather than the com-

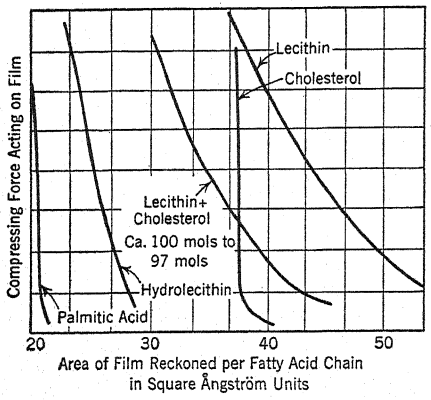


FIG. 56.—Pressure-area curves of palmitic acid, cholesterol, and the fatty acids in the lecithin molecule.

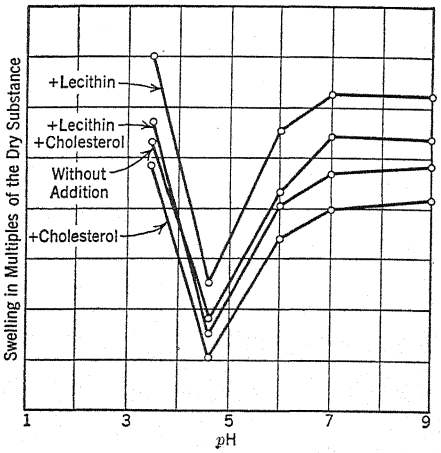


FIG. 57.—Effect of cholesterol and lecithin on the swelling of gelatin.

plex polar group, and therefore again resulting in a change in the cohesive forces of the carbon chains. Just what physiological significance may be attached to such physical phenomena must be the burden of further research, as they undoubtedly touch the very foundation of physiology, though not that science alone.

Bamberger[28] has made some interesting studies on the influence of lecithin and cholesterol on the swelling and bound water relations of gelatin. Fig. 57 shows the swelling of gelatin as a function of *p*H, with and without the addition of lecithin and cholesterol as well as with these two together. The lecithin concentration was 5 per cent and that of

[28] P. Bamberger, Biochem. Z. **266**, 175 (1933).

cholesterol 0.5 per cent on a dry basis. Bamberger calculates that a lecithin molecule is about fifty times more active than a hydrogen ion. The increase in water uptake of the gelatin by lecithin and the corresponding decrease in the uptake by cholesterol must be due to the effect of these substances on the gel structure and really has nothing to do with the bound water or water of hydration as shown in Table XXIII. The lecithin increases the imbibition of water but decreases the "bound" water.

TABLE XXIII

"BOUND" WATER IN PER CENT OF THE TOTAL WATER OF THE GEL *p*H 6.9 (19 HOURS AT −22° C.)

Total Water	Gelatin	Gelatin + Glycogen	Gelatin + Lecithin	Gelatin + Cholesterol
80	12.4	11.4	10.2	11.8
67	24.2	24.2	23.0	24.1
50	50.0	50.1	49.0	51.2

Strangely enough, Moraczewski and Sadowski[29] obtained completely opposite results from those of Bamberger. They found cholesterol to promote and lecithin to inhibit the swelling of gelatin. Neither Bamberger nor Moraczewski and Sadowski prepared their own lecithin but depended on a commercial source of supply. This whole important question should be reinvestigated using chemicals of undoubted purity.

Hughes[30] has studied the surface potential of monomolecular films of lecithin and other substances on permanganate solutions. His findings are in substantial agreement with those of Leathes. Fig. 58 shows some of his results. The change in surface potential is evidently a measure of the degree of oxidation. Triolein has an area of 44 sq. Å. per hydrocarbon chain, which is too small to permit the presence of the unsaturated double bonds in the aqueous surface and oxidation is inhibited. Lecithin is attacked rapidly, whereas lysolecithin which has no unsaturated fatty acid is acted upon more slowly. Incidentally, there must be considerable difference in the manner of orientation between the α- and β-lecithin, and it would be expected that they would give rather different surface areas. Experiments along this line should be conducted.

Physiology of the Phospholipids. At this point we will turn our attention briefly to a consideration of the physiology of the phospholipids.

[29] W. V. Moraczewski and T. Sadowski, Biochem. Z. **276**, 388 (1935).
[30] A. Hughes, Biochem. J. **29**, 430 (1935).

The phospholipids correspond roughly to the so-called *élément constant* (*vide infra*). Sinclair[31] has written an excellent review of this subject. Since phospholipids seem to have a natural occurrence in all living cells, the question arises as to their function. The answer to this question is a challenge to the biochemist, and as yet response has been very inadequate. Three functions have been proposed for the phospholipids: (*a*) that they are metabolic intermediates and that it is necessary to convert the neutral fat into phospholipid (or at least lecithin) before it can be metabolized; (*b*) that they act as oxygen carriers owing to the presence of their easily oxidizable double bonds; and (*c*) that they constitute part of the structure of protoplasm.

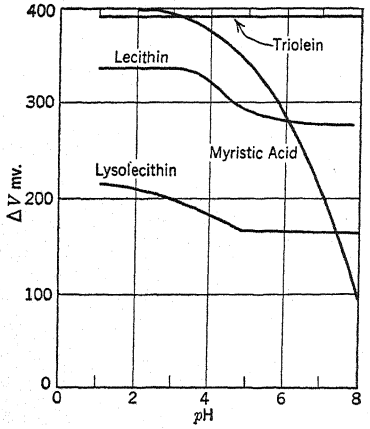


FIG. 58.—Surface potentials of films on permanganate solutions at 20° C; lecithin at 100 sq. Å; lysolecithin at 100 sq. Å; triolein at 95 sq. Å; mytistic acid at 20 sq. Å.

Tait and King[32] investigated the rate of oxygen uptake by lecithin in the presence of glutathione and found it to be about four times larger than the rate of oxygen uptake by the fatty acids derived from the hydrolysis of the lecithin and to be very much larger than the rate of oxidation of the hydrolyzed constituents of lecithin, thus indicating that the structure of the lecithin molecule enhances the oxidizability of the fatty acids it contains.

Fat has a dual rôle in metabolism. A portion is used in the synthesis of certain essential constituents of the tissue. The remainder serves as fuel which can be utilized immediately or stored for the future. These two kinds of fat have been given appropriate names, the so-called *élément constant* and *élément variable*. The tissues of animals that have been starved to death still contain a certain amount of fat which seems to be fairly constant for any given species. This presumably consists of phospholipids, etc.

Fasting affects the phospholipid content of some organs but not of others. Cahn and Bonot[33] found that after dogs had been starved to a 30 per cent decrease in weight the phospholipid was higher than normal

[31] R. G. Sinclair, Physiol. Rev. **14**, 351 (1934).
[32] H. Tait and E. J. King, Biochem. J. **30**, 285 (1936).
[33] T. Cahn and A. Bonot, Am. physiol. physiochem. biol. **4**, 485 (1928).

in the liver and lungs and probably in the kidneys. It was about normal in the brain and intestine and about 20 per cent below normal in the muscles. Thus it is seen that the phospholipid corresponds to the élément constant only for certain organs. The phospholipids should not be considered in general as stored material. It appears that the kidney, spleen, lung, and heart contain no élément variable but only élément constant. For example, in overfed, normal, and starved animals the following tissues had the recorded percentages of fatty acids.

	Overfed	Normal	Starved
Kidney.....	11.1	11.9	13.4
Muscle.....	17.6	11.3	4.6
Liver.....	12.9	10.5	11.3

The phospholipid content varies greatly from organ to organ. Bloor[34] found for the various fresh organs of beef the following percentages of phospholipid (lecithin + cephalin): brain, 4.58; liver, 3.06; pancreas, 1.86; kidney, 1.62; lung, 1.25; heart muscle, 1.64; jaw muscle, 1.06; diaphragm, 0.76; neck muscle, 0.63; round muscle, 0.42. The white matter of the brain was found to contain most of the phospholipid of that organ. Bloor believes that the phospholipid content of the muscles increases in the same order as their activity.

Sinclair[35] has recently studied the passage of elaidic acid into tissue phospholipids of the rat. He found that rats raised on a diet rich in trielaidin grew normally and that the elaidic acid was incorporated in the phospholipid to the extent of about a third of the total phospholipid fatty acid. When trielaidin is fed to adult rats, the rate of entrance of elaidic acid into and the rate of disappearance from the phospholipid is rapid in the liver and slow in the muscle. The passage of elaidic acid into the phospholipids of the liver is complete in one day but takes several days in the muscle. From these findings, Sinclair argues for two classes of phospholipids, one which has to do with the protoplasmic structure and accordingly does not undergo rapid changes, and the other which takes part in the tissue metabolism and in which there is a rapid turnover. Sinclair[36] has confirmed these findings with similar studies on the cat. Within a few hours after feeding trielaidin, (*a*) the phospholipids of the intestinal mucosa contained large amounts of elaidic acid, thus confirming the rapid taking-up of ingested acids previously deduced from the change in the degree of unsaturation; (*b*) as much as 28 per cent of the fatty acids in the phospholipids of the

[34] W. R. Bloor, J.B.C. **68**, 33 (1926); **72**, 327 (1927); **80**, 443 (1928).
[35] R. G. Sinclair, J.B.C. **111**, 515 (1935).
[36] R. G. Sinclair, J.B.C. **114**, XCIV (1936).

blood plasma consists of elaidic acid, thus confirming in a very direct way the long-assumed transport function of plasma phospholipid; (*c*) no elaidic acid was detected in the phospholipids of the red blood cells, indicating that, in the cat, they do not participate in fatty-acid transport; (*d*) the phospholipids of the liver contain considerable amounts of elaidic acid and therefore undergo a rapid turnover; (*e*) the kidney phospholipids contain no elaidic acid, indicating a slow rate of turnover and thus a non-metabolic function. Sinclair has certainly devised a very ingenious and powerful method of attack, which together with Schoenheimer's method of tagging a molecule with deuterium, should lead finally to a solution of many of our problems of the chemistry of the metabolism of the lipids.

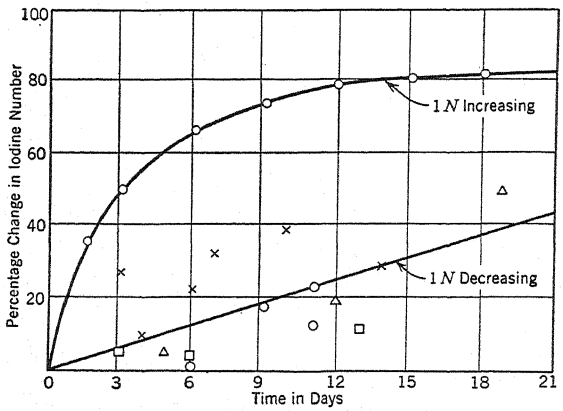


FIG. 59.—The rate of decrease in the iodine number of the phospholipid fatty acids in the carcass and muscle as a result of fasting or of feeding hydrogenated cocoanut oil. × represents the carcass, ○ the muscle of fasting rats; □ represents the carcass, △ the muscle, of rats fed hydrogenated cocoanut oil. The upper curve shows, for comparison, the rate of increase in the iodine number of the phospholipid fatty acids in the carcasses of rats after changing from a fat-poor diet to one rich in cod-liver oil.

Sinclair[37] has pointed out several times that it is quite difficult to replace the unsaturated fatty acids of the phospholipid with more saturated ones. On the other hand, phospholipid will pick up the unsaturated fatty acids with great avidity. Fig. 59 shows the percentage of decrease of the iodine number of the fatty acids of the phospholipids of the muscle and carcasses of rats fed on hydrogenated cocoanut oil. Likewise the percentage of increase of the iodine number upon feeding is indicated. This demonstrates the remarkable "hunger" that phospholipid has for unsaturated fatty acids. Strangely enough, however, as was indicated elsewhere, Sinclair[38] has been able to show that the ratio of the saturated to unsaturated fatty acid in the phospholipid is constant. The increase in iodine number comes in the extent of unsaturation of the unsaturated fatty acids, but the amount of unsaturated acid remains constant.

[37] R. G. Sinclair, J.B.C. **111**, 275 (1935).
[38] R. G. Sinclair, J.B.C. **111**, 261 (1935).

Much has been made of the phospholipid-cholesterol ratio. In general there is a close relation between the free cholesterol and the phospholipid; if one increases the rise is followed closely by the other lipid. It is believed that the ratio has much to do with the water balance of the body. This theory was put forward by Schaeffer[39] and elaborated on with *in vitro* experiments by Degkwitz.[40] Lecithin was found to enhance greatly the water-imbibing power of gelatin whereas cholesterol diminished this power. It was believed, therefore, that a tissue with a high phospholipid-cholesterol ratio would imbibe a great deal of water, and conversely. Degkwitz believes that his *in vivo* experiments confirmed his theory. The results of Schaeffer, however, showed that, if the tissues were immersed in water under standard conditions, the imbibition proved to be inversely proportional to the phospholipid-cholesterol ratio. It should be added, in regard to Degkwitz's results, that gelatin is an unfortunate protein from which to generalize. It has a gel structure which holds water in an entirely different way from other proteins which do not form gels. A low phospholipid-cholesterol ratio would favor a water-in-oil emulsion which might greatly enhance the water uptake by the adipose tissue. This point should certainly be considered in any theory of the water balance of the body.

Sinclair emphasizes, and rightly, our lack of knowledge concerning the rôle of the individual phospholipids. We have grouped them all under the heading "phospholipid" and have not differentiated between the individual phospholipids. There are three main classes of phospholipids, the lecithins, the cephalins, and the sphingomyelins. Each class exhibits many isomers. For example, lecithin occurs in the symmetrical (β) and the unsymmetrical (α) form. In addition, there are many isomers, resulting from the different fatty acids present as well as the two positions available for these fatty acids in the α-lecithin. Only in the mechanism of blood clotting has an individual phospholipid been assigned a specific rôle. Here cephalin is believed to play a very important part, while lecithin is inactive.[41]*

LECITHO PROTEINS

Sørensen[42] has discussed the problem of the lecitho proteins. The phospholipid seems to be always associated with the globulin fraction, wherever it occurs in a mixture of proteins. In fact, Miss Chick says that euglobulin in serum is a complex material, formed from pseudo globulin by association with some serum lipid, and that the phosphorus

[39] P. A. Schaeffer, J. physiol. path. gén. **16** (1914).

[40] R. Degkwitz, Lipoid und Ionen, Theodor Steinkopff, Dresden, 1933.

[41] G. R. Minot and R. I. Lee, Arch. Internal. Med. **18**, 474 (1916); H. Bull, Johns Hopkins Hospital Bull. **42**, 199 (1928).

[42] S. P. L. Sørensen, Kolloid.-Z. **53**, 306 (1930).

* See page 156 for additional discussion of the physiology of the phospholipids.

content of the euglobulin comes from the phospholipid. She claims to have prepared euglobulin from aqueous lecithin emulsion and salt-free pseudo globulin. Sørensen believes that the union between the phospholipids and globulin, as well as with serum albumin, is a very loose one. He believes also that the fact that a certain fraction separates out when the native serum is treated with certain reagents is no proof that these fractions actually exist in nature. Sørensen, himself, was never able to combine pure serum powder and phospholipid extracted from serum. He points out that only about one-fifth of the phospholipid of serum is extractable with ether, indicating that most of the lipid is not in a free condition. This is true of other proteins; for example, Blackwood and Wishart[43] found that about 2 per cent of the lecithin of egg yolk is extremely closely bound to the vitellin molecule.

The egg proteins represent[44] much the same situation as is found with the serum proteins. There are apparently two well-defined egg-yolk proteins: vitellin and livetin. The vitellin carries with it the phospholipid and behaves as a globulin; livetin has no phospholipid and has the characteristics of a pseudo globulin. Vitellin slowly loses its phospholipid in contact with water, and thereby becomes more and more insoluble in salt solutions. In short, it seems to owe its globulin character to its phospholipid content.

Mills[45] has found that tissue contains a substance which accelerates the clotting of blood, and that this substance is composed of a protein and phospholipid in the ratio of approximately 58.4 to 41.6 per cent, respectively. A large part of the phospholipid can be removed by extraction with fat solvents. A part, however, is very firmly held and is not completely removed, except by methods which tend partially to hydrolyze the protein.

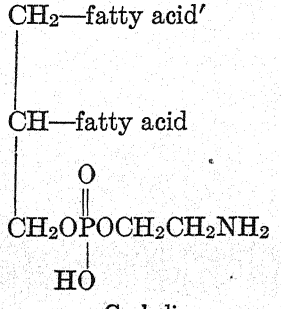
Horsfall and Goodner[46] have recently studied the relation of the lipids to agglutination and precipitation. They found that the removal of lipids from type 1 antipneumococcus horse serum causes a loss of the visible phenomena of type-specific agglutination and precipitation and, in rabbit serum, a marked reduction in these properties. Initial activity of the type-specific antibody can be restored to extracted horse serum by the addition of lecithin, and to rabbit serum by the addition of cephalin. These are very important findings and seem to indicate that the phospholipids play an important rôle in specific reactions. Naturally this rôle is played in connection with protein since there is nothing specific about a phospholipid alone.

[43] J. H. Blackwood and G. M. Wishart, Biochem. J. **28**, 550 (1934).
[44] T. H. Jukes and H. D. Day, J. Nutrition **5**, 81 (1932).
[45] C. A. Mills, Am. J. Med. **172**, 501 (1926).
[46] F. L. Horsfall, Jr., and K. Goodner, J. Exptl. Med. **62**, 485 (1935).

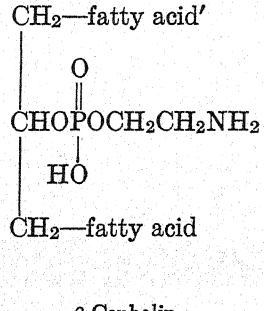
De Jong and Westerkamp[47] studied mixtures of lecithin and various proteins. They found that lecithin and protein form a complex in the *p*H range between their isoelectric points but at no other hydrogen-ion concentration, that is to say, only when the lecithin and protein molecules have opposite electrical charges. The isoelectric point of the complex varied between the isoelectric point of the lecithin which they used (2.7) and that of the protein, depending upon the relative concentration of the two constituents. The compound had all the earmarks of an adsorption complex involving no definite chemical compound. De Jong and the Kruyt school call such complexes "coacervates." It must be added, however, that it is questionable if these artificial combinations have any significance so far as the natural products are concerned. At a *p*H 7, which is approximately the reaction of tissue, all proteins except certain very unusual ones are negatively charged. The isoelectric point of pure lecithin as experimentally determined is 6.7, but from theoretical considerations Chain and Kemp believe it to be 7.5. Bull and Frampton have shown, however, that small amounts of cephalin greatly lower the isoelectric point of lecithin, which lowering would not permit a coacervate to form between lecithin and protein. Incidentally, in this work of de Jong and Westerkamp, the isoelectric point of the lecithin used was 2.7, which indicates either that considerable cephalin was present, or else that extensive hydrolysis of the fatty acids from the lecithin had taken place. A point which is not always appreciated in a study of the lecitho proteins is that seldom is any identification of the phospholipid involved. A better name would be phospho-lipo-proteins.

CEPHALIN

The structure of cephalin is identical with that of lecithin except that it has β-amino ethyl alcohol (cholamin) in place of choline. Cholamin is a very weak base and is unable to compensate for the strong phosphoric acid residue which gives to cephalin an acid character with a very low isoelectric point. The fatty acids reported are stearic, oleic, linoleic and arachidonic.



α-Cephalin



β-Cephalin

[47] H. G. Bungenberg de Jong and R. F. Westerkamp, Biochem. Z. **234**, 367 (1931).

Cephalin may be prepared by the method of Levene and Rolf,[48] who allowed an ether solution of brain lipids to stand in the cold. The sphingomyelin and cerebrosides precipitated out. The cephalin was separated from the lecithin by treating the ether-soluble material with alcohol. The cephalin is insoluble in alcohol. The precipitated cephalin is dispersed in water and then precipitated with the addition of hydrochloric acid. This material is still further purified. Levene, in one experiment, obtained 18 grams of purified cephalin from 18 kg. of brain tissue.

Cephalin, in order to be considered pure, must have a nitrogen to phosphorus ratio of one. All the nitrogen must analyze as amino nitrogen, and it must be free from oxidation as indicated by its lack of yellow color. The β-amino ethyl alcohol does not give good, insoluble, well-defined salts, as does choline, so that it is difficult to estimate. The estimation is usually made by means of the Van Slyke amino nitrogen method, after hydrolysis and separation of the fatty acids.

Cephalin, in contrast to lecithin, precipitates out of its solutions as a powder-like material. It is insoluble in alcohol, in acetone, and in completely anhydrous ether, but if the ether contains moisture, it is entirely soluble. It dissolves in petroleum ether, chloroform, carbon disulfide, benzene, and hot acetic acid. In the presence of lecithin, it has appreciable solubility in ethyl alcohol, and, indeed, considering the wide difference between the isoelectric point of lecithin and that of cephalin, the two should form coacervates over a wide range of hydrogen-ion concentration. Cephalin becomes hydrated to build myelin forms. The water emulsions can be precipitated with hydrochloric and other mineral acids. The cadmium chloride double salt contains less cadmium chloride than would correspond to a molecular compound. The same is true of the platinum chloride compound. Cephalin crystallizes out of pyridine in snow-white needles, with a melting point of 174° C. Hydrogenation does not proceed as easily as it does for lecithin. Cephalin quickly oxidizes in the presence of air, becoming dark in color.

Grün and Limpächer[49] claim to have synthesized cephalin. The same technique was used as in the synthesis of lecithin.

Magistris[50] reports an extensive study on lysolecithin and lysocephalin. His lysocephalin showed very little hemolytic power, and he goes so far as to say that he believes it possible that lysocephalin completely freed from lysolecithin is entirely devoid of hemolyzing power.

Spiegel-Adolf[51] has studied the protective power of cephalin. She

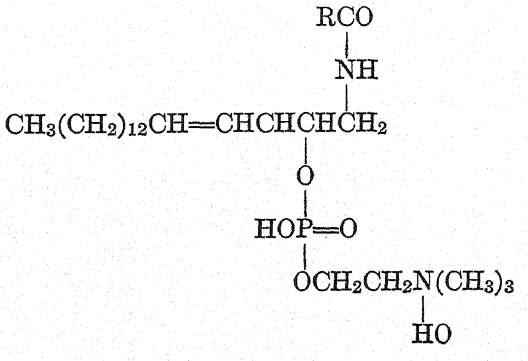
[48] P. A. Levene and I. P. Rolf, J.B.C. **74**, 713 (1927).
[49] A. Grün and R. Limpächer, Ber. **60B**, 1, 151 (1927).
[50] H. Magistris, Biochem. Z. **210**, 85 (1929).
[51] Mona Spiegel-Adolf, J.A.C.S. **57**, 1431 (1935).

found cephalin to protect colloidal gold three or four times more effectively than lecithin did. Cephalin is not flocculated by serum albumin, and she found that one part of cephalin is able to prevent heat denaturation of two parts of serum albumin. She prepared her cephalin from human brains. Cephalin is supposed to play an important rôle in blood clotting.

SPHINGOMYELIN

Sphingomyelin is distinguished from lecithin and cephalin by its insolubility in ether and by the absence of glycerol in the molecule. It is found in large quantities in the brain and nerves but also in smaller amounts in many other organs. Sphingomyelin constitutes, along with the cerebrosides, the main part of the so-called protagon which was for a time considered to be a chemical compound and found later to be a mixture.

Sphingomyelin can be prepared by the method of Levene[52] which makes use of the solubility of sphingomyelin in hot alcohol, pyridine, and acetic acid, and its insolubility in cold alcohol, ether, and acetone. The yields are very small. The compound is white and crystalline, nonhydroscopic, and stable in air and light. It becomes hydrated and emulsifies in water. It yields a cadmium chloride double salt and shows optical activity. It contains a fatty acid, phosphoric acid, and two nitrogen bases, choline and sphingosin. This last is a C_{18} unsaturated amino alcohol. The constitution of sphingomyelin is probably



The position of the hydroxyl group in the sphingosin is not definitely established, nor is the place of attachment of the phosphoric acid on the sphingosin certain. The compound is hydrolyzable with $Ba(OH)_2$ or H_2SO_4. At least three fatty acids, stearic, lignoceric, and nervonic, have been reported in sphingomyelin. Chain and Kemp[53] find the

[52] P. A. Levene, J.B.C. 24, 69 (1916).
[53] E. Chain and I. Kemp, Biochem. J. 28, 2052 (1934).

isoelectric point of sphingomyelin to be greatly dependent on impurities but report the isoelectric point probably to be about *p*H 7.0, as for lecithin.

In Niemann-Pick's disease, there is an accumulation of sphingomyelin in the brain, liver, and spleen, the other phospholipids remaining normal. The cerebrosides are absent. Klenk[54] suggests, on the basis of observations made on post mortem analysis of a subject suffering from this disease, that sphingomyelin is an intermediate in lipid metabolism. Niemann-Pick's disease is always associated with amaurotic idiocy.

[54] E. Klenk, Z. physiol. Chem. **235**, 24 (1935).

CHAPTER VII

CEREBROSIDES

There has been much confusion regarding the cerebrosides. It was thought at one time that "protagon" was a definite entity. It has since been shown that it is a mixture of cerebrosides and phospholipids and separates with the appearance of a chemical compound owing to the mutual solubility of these materials. Four cerebrosides are recognized at present: cerebron (phrenosin), kerasin, nervon, and oxynervon.

The cerebrosides are made up of galactose, a higher fatty acid, and a base, sphingosin. As stated above, four have been identified. Cerebron and kerasin have been known for many years. Nervon was prepared by Klenk[1] in 1925, and at the same time he identified oxynervonic acid and so concluded that there must be another cerebroside, oxynervon. Thierfelder does not believe that there are other cerebrosides.

The cerebrosides are found in the white substance of nerve cells and are probably a general component of all cells. They have been found in fungi, in oak wood, and in seeds. They contain carbon, oxygen, hydrogen, and nitrogen. In general they exist in the amorphous state but have the property of forming liquid crystals. The cerebrosides are insoluble in water as well as in ether and petroleum ether but are soluble in warm alcohol and in pyridine at ordinary temperatures as well as in many other organic solvents. They are optically active.

Klenk and Levene have engaged in an extended controversy concerning the fatty acids in cerebron. Klenk maintains the acid to be a twenty-four-carbon hydroxy acid, to which the name cerebronic acid was assigned. Levene, on the other hand, believes the acid to have the empirical formula $CH_3(CH_2)_{22}CHOHCOOH$. The occurrence of a fatty acid with an odd number of carbon atoms would be very unusual. Chibnall, Piper, and Williams[2] studied the question with X-ray technique. They came to the conclusion that cerebronic acid was a mixture of C_{22}, C_{24}, and C_{26} α-hydroxy acids but denied the presence of an uneven number of carbon atoms in the chain. They likewise analyzed lignoceric acid and found it to be made up of C_{22}, C_{24}, and C_{26} saturated acids. It appears, therefore, that the cerebrosides may be

[1] E. Klenk, Z. physiol. Chem. **145**, 244 (1925).
[2] A. C. Chibnall, S. H. Piper, and E. F. Williams, Biochem. J. **30**, 100 (1936).

more complex than first supposed. It is indeed strange that other acids such as stearic and palmitic are not found.

The cerebrosides have been assigned the following constitution:

Fatty acid—Sphingosin—Galactose

The cerebrosides differ from each other only in their fatty acids. According to Klenk the fatty acids bear a simple relation to one another. Lignoceric acid, a saturated acid found in kerasin and sphingomyelin,

$$CH_3(CH_2)_7CH_2CH_2(CH_2)_{12}CH_2COOH$$

Cerebronic acid or hydroxy lignoceric acid, found only in cerebron,

$$CH_3(CH_2)_7CH_2CH_2(CH_2)_{12}CHOHCOOH$$

Nervonic acid, an unsaturated lignoceric acid,

$$CH_3(CH_2)_7CH{=}CH(CH_2)_{12}CH_2COOH$$

Oxynervonic acid, an unsaturated hydroxy lignoceric acid,

$$CH_3(CH_2)_7CH{=}CH(CH_2)_{12}CHOHCOOH$$

As indicated in the above paragraph, this list of fatty acids must be accepted with caution.

Sphingosin is an unsaturated amino alcohol and is supposed to have the following structure:

$$CH_3(CH_2)_{12}CH{=}CHCHOHCH_2CH_2NH_2$$

Galactose is an aldo hexose.

The preparation and purification of the cerebrosides are very involved; the reader should consult "Die Chemie der Cerebroside und Phosphitide" by Thierfelder and Klenk for details. This book also contains a wealth of information concerning the chemistry of those substances. The following is a brief outline of the method employed by Rosenheim[3] for the preparation of cerebron and kerasin. The material (brain tissue) is treated with acetone and extracted first with petroleum ether and then with hot pyridine to dissolve the cerebrosides, which are then precipitated by the addition of acetone. The precipitate is extracted in a soxhlet with ether. The residue is about 2 per cent of the fresh brain. The residue is treated with hot acetone containing 10 per cent water and filtered. It is then allowed to stand for sixteen hours at 37° C. The cerebron separates out of solution. The filtrate is then cooled in an icebox for twenty-four hours, whereupon the kerasin is precipitated. The two fractions are purified.

[3] O. Rosenheim, Biochem. J. 8, 110 (1914).

Nervon was prepared by Klenk.[4] Ninety-seven cow brains were hashed and the ether extract treated with acetone. The precipitate was dissolved in petroleum ether and again precipitated by acetone, then placed in a large mortar and mixed with 96 per cent alcohol. Lead acetate in an ammonium solution of methyl alcohol was added. The precipitate was suspended in hot 96 per cent alcohol and H_2S passed in.

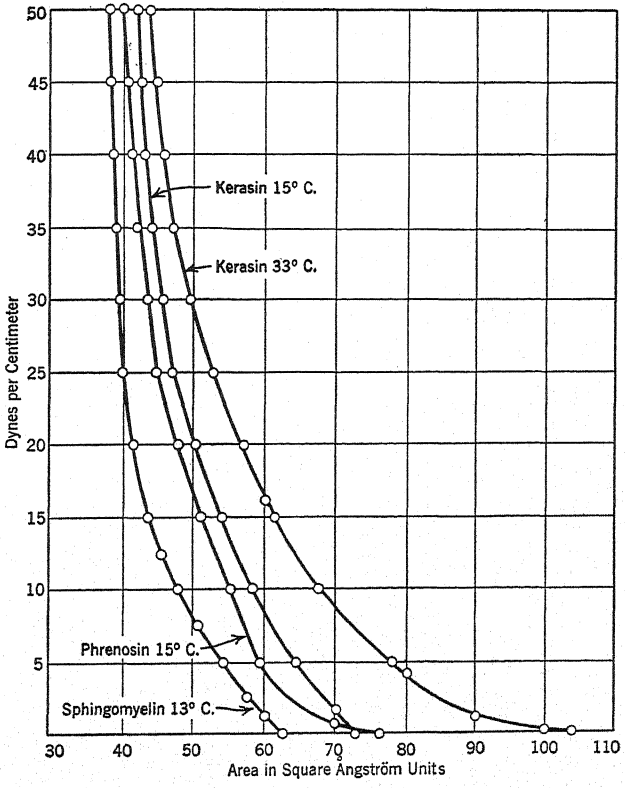


FIG. 60.—Pressure-area curves of sphingomyelin, kerasin, and phrenosin.

The filtrate was cooled in an icebox and the precipitate shaken with acetone. The precipitate was dissolved in chloroform-methyl alcohol (1 : 3), cooled, and filtered. The resulting precipitate was dissolved in hot methyl alcohol, and cadmium acetate in methyl alcohol solution was added. The precipitate was filtered and the filtrate treated with H_2S, cooled, and the precipitate crystallized from acetone pyridine (1 : 1).

[4] E. Klenk, Z. physiol. Chem. 145, 244 (1925).

Nervon is a white and crumbly mass; it has much the same solubilities as the other cerebrosides, especially kerasin. It melts at 180° C., is levorotory, and has an iodine number of 62.7.

Turner and Watson[5] studied surface films of sphingomyelin, cerebron, and kerasin on water. Fig. 60 shows their results. The final area of about 42 sq. Å. for the high pressure is surprisingly small as it is about the area that would be taken up by the paraffin chains in the molecule if these were vertical. The films are liquid at ordinary temperatures.

TABLE XXIV

SOLUBILITIES * OF PHOSPHOLIPIDS AND CEREBROSIDES IN VARIOUS SOLVENTS

Material	Ether	Alcohol	Acetone	Petroleum Ether	Pyridine	Water	$CH_3OH + CHCl_3$
Lecithin.....	S	S	X	S	.....	X	
Cephalin.....	S	X	X	S	.....	X	
Sphingomyelin...	X	H	X	.....	H	X	S
Cerebron.....	X	H	H	X	S	X	S
Kerasin.....	X	H	H	X	S	X	S
Nervon.....	X	H	H	X	S	X	S

* The solubilities indicated are of a purely relative nature. S means soluble, X insoluble, and H soluble in hot solvent.

Thannhauser and Reichel[6] report that cerebrosidase is practically inactive but is activated in the presence of H_2S, cysteine, -SH glutathione, and l-ascorbic acid. The substances which stimulate cerebrosidase activity depress the activity of the sphingomyelinase. They point out the importance of their studies in relation to the pathogenesis of Gaucher's disease on one hand and Niemann-Pick's disease and amaurotic idiocy on the other.

[5] K. Turner and M. M. Watson, Biochem. J. **24**, 113 (1930).
[6] S. J. Thannhauser and M. Reichel, J.B.C. **113**, 311 (1936).

CHAPTER VIII

CARBOHYDRATE ESTERS OF THE HIGHER FATTY ACIDS

Sugar Esters of Fatty Acids. Bloor[1] attempted to follow digestion by feeding an optically active synthetic fat. It is usually considered that natural triglycerides should contain an asymmetric carbon atom and should accordingly be optically active, but none have been found to show optical activity. Bloor synthesized the distearic acid ester of mannitol. In the course of his synthesis, he obtained several closely allied compounds, all of which were optically active. The method of preparation was to dissolve mannitol in concentrated sulfuric acid at 70° C., add the stearic acid, and keep the mixture at 70° C. for three to four hours. The distearate was extracted with ether. In this way he obtained mannitan distearate and mannid distearate. If the mannitan distearate is heated to 200° C. it is transformed into the isomannid distearate.

Bloor found that mannid distearate, with human pancreatic juice, shows a digestibility of about one-third that of cotton oil; with castor bean lipase, about one-half that of cotton oil. Mannitan distearate does not seem to be attacked by the lipase of the castor bean, but with the various pancreas preparations a digestibility of one-fourth to one-half that of cotton oil was obtained. Feeding experiments bore out these conclusions. Mannitan dilaurate as well as isomannid dilaurate were also prepared.[2] The products obtained greatly resembled the homologous stearic acid compound. The isomannid esters of lauric and closely related fatty acids were as well utilized by the animal organism as ordinary fats were.

Hess and Messmer[3] were able to synthesize numerous sugar esters of the fatty acids. They treated the sugar with the acid chloride in the presence of pyridine. The pentapalmityl-, pentastearyl-, pentaollyl-glucoses and the octapalmityl-, and octastearyl-saccharoses and the hendecapalmityl- and hendecastearyl-raffinoses have the same physical properties as ordinary glycerol fat.

Schmaltz[4] has studied the colloidal properties of glucose mono-

[1] W. R. Bloor, J.B.C. **11**, 141 (1912).
[2] W. R. Bloor, J.B.C. **11**, 429 (1912).
[3] K. Hess and E. Messmer, Ber. **54B**, 499 (1921).
[4] D. Schmaltz, Kolloid-Z. **71**, 234 (1935).

stearate prepared by the above method of Hess and Messmer. He found the compound to be a waxy solid which forms thixotropic gels in paraffin oil, and he also found it to be an excellent emulsifying agent for the water-in-oil type of emulsion.

Starch Esters of Fatty Acids. Taylor and co-workers have investigated the naturally occurring combination between fatty acids and starch. Taylor and Nelson[5] first showed that a small percentage of fatty acids are bound very tightly to starch. Taylor and Lehrman,[6] even after the most thorough extraction of corn starch with fat solvents, found that 0.5 to 0.6 per cent fatty acids were obtained upon hydrolyzing the starch. The fatty acids are found associated only with the α-amylose fraction and not at all with the β-amylose. The fatty acids consisted of 24 per cent palmitic, 40 per cent oleic, and 36 per cent linoleic. Taylor and Sherman[7] studied the splitting of the fatty acids from the corn starch by means of acids, bases, and amylase. They found that the unsaturated acids were always hydrolyzed off more easily than the saturated ones. Lehrman[8] reports similar studies on rice starch, and later on wheat starch. P. Koets, of Utrecht, Holland, has suggested to the author the interesting possibility that the fatty acids associated with starch really occur as a phospholipid in combination with the starch. In this connection Przylecki and Majmin[9] investigated the combination formed when lecithin is added to starch and similar compounds. They report that about 50 molecules of lecithin combine with one molecule of starch. They name such a combination a "symplex." The lecithin-glycogen symplex contains 60 per cent

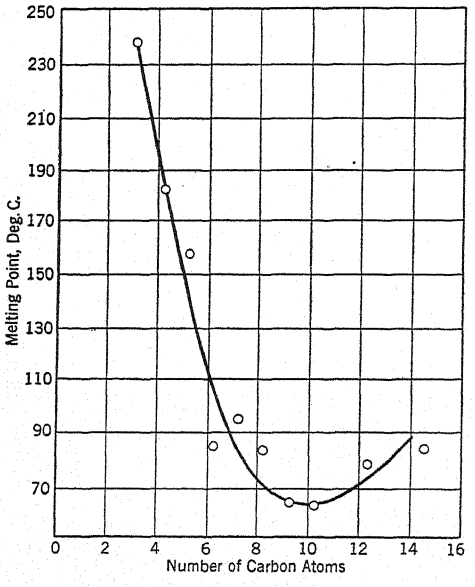


FIG. 61.—Melting points of the cellulose esters of the fatty acids.

[5] T. C. Taylor and J. M. Nelson, J.A.C.S. **42**, 1726 (1920).
[6] T. C. Taylor and L. Lehrman, J.A.C.S. **48**, 1739 (1926).
[7] T. C. Taylor and R. T. Sherman, J.A.C.S. **55**, 258 (1933).
[8] L. Lehrman, J.A.C.S. **51**, 2185 (1929); **52**, 808 (1930).
[9] St. J. V. Przylecki and R. Majmin, Biochem. Z. **280** 413 (1935).

glycogen and is easily soluble in water. The lecithin-dextrin symplex is also very soluble and contains about four lecithin molecules to one dextrin. Unfortunately in these studies they used commercial lecithin, the purity of which cannot be relied upon.

Cellulose Esters of Fatty Acids. Sheppard and Newsome[10] investigated the density, melting points, moisture regain, work of adhesion, and spreading of the tricellulose esters of the fatty acids from acetic through myristic. They found the density curve to be much the same as that for the fatty acids, but no step-like course is exhibited. The melting-point curve is shown in Fig. 61, and as can be seen, the typical alternation between even and odd numbers is not present. N. K. Adam[11] has reported surface-area measurements on fatty acid-cellulose esters; cellulose acetate is of tremendous commercial importance. So far as the author is aware, these fatty acid-cellulose esters have no natural occurrence.

[10] S. E. Sheppard and P. T. Newsome, J. Phys. Chem. **39**, 143 (1935).
[11] N. K. Adam, Trans. Faraday Soc. **29**, 90 (1933).

CHAPTER IX

EMULSIONS

A liquid dispersed in another liquid with which it is ordinarily immiscible is called an emulsion. Emulsions are usually thermodynamically unstable, and experimentally an emulsion will break or cream after a time. If we equate the surface energy decrease experienced by two cubical particles which coalesce (this represents the energy tending to produce coalescence) to the kinetic energy of the particles (which is the energy tending to keep the particles apart) we have for a stable emulsion:

$$2\gamma r^2 \leqq \tfrac{3}{2}KT$$

where γ is the interfacial tension; r, the radius of the particle; K, the Boltzmann constant; and T, the absolute temperature. In Table XXV, T has been assumed to be equal to 300°, and values of r calculated as the maximum radius which will yield a stable suspension for the given interfacial tensions. It is apparent that the interfacial tension must be greatly reduced before a completely stable emulsion is obtained.

TABLE XXV

RADII WHICH WILL YIELD A STABLE SUSPENSION FOR A GIVEN INTERFACIAL TENSION

Interfacial Tensions in Dynes per Centimeter	r in Centimeters
100	0.176×10^{-7}
20	0.393×10^{-7}
5	0.785×10^{-7}
1	1.76×10^{-7}
0.5	2.48×10^{-7}
0.1	5.56×10^{-7}
0.01	17.6×10^{-7}

In addition to the kinetic energy which tends to keep the particles apart, there is also the electrostatic charge on the particles. The electrical effects are of less importance in emulsions than they are in other colloid systems, because the particles are much larger in emulsions, and also because usually an emulsion micelle is protected by an emulsifier which

is lyophilic, which tends to outweigh the electrical effects in so far as stability is concerned.

Since an emulsion in its simplest form is a mixture of two mutually insoluble liquids, it is clear that two kinds of emulsions are possible, depending upon which liquid forms the dispersed phase and which the dispersion medium. When water is one of the components, we speak of these two types of emulsions as oil-in-water or water-in-oil emulsions. Emulsions of biological interest almost invariably have water as one of the phases.

It is difficult but not impossible to produce emulsions containing one pure liquid dispersed in another pure liquid. With the aid of ultrasonic waves it is thus possible to produce emulsions with very small particles of mercury in water, which are fairly stable.[1] By the same technique, paraffin oil can be suspended in water. The stability of these suspensions depends upon the particle size being very small.

Emulsifiers. Most emulsions are stabilized by the presence of a third substance usually spoken of as a stabilizer or emulsifier. Some emulsifiers favor an oil-in-water, others, a water-in-oil, emulsion. It is a general rule that hydrophilic stabilizers produce emulsions of the oil-in-water type, whereas hydrophic stabilizers produce emulsions of the water-in-oil type. It is a general but not necessary condition that the emulsifier be in the colloidal state; sometimes emulsifying agents are solid powders.

Degkwitz[2] gives a classification of emulsifiers, according to the type of emulsion favored by the emulsifier. His classification is shown in Table XXVI. Although the emulsifying agent controls to a great extent the kind of emulsion to be expected, the type of oil is also impor-

TABLE XXVI

CLASSIFICATION OF EMULSIFIERS

Oil-in-water	Water-in-oil
Protein	Cholesterol
Lecithin	Cholesterol esters
Sphingomyelin	Cephalin
Glycogen	Cerebron
"Monovalent" soaps	H^+
OH^-	Ca^{++}
Cl^-	Mg^{++}
PO_4^{---}	Fe^{+++}
SO_4^{--}	

[1] H. B. Bull and K. Söllner, Kolloid-Z. 60, 263 (1932).
[2] R. Degkwitz, Lipoide und Ionen, Theodor Steinkopf, Dresden, 1933.

TABLE XXVII

TYPE OF EMULSION AND SPECIFIC GRAVITY OF OIL

Name of Oil	Specific Gravity	Type of Emulsion *	Stability
Isohexane	0.664	O–W	Stable
Isooctane	0.726	O–W	Stable
Kerosene	0.820	O–W	Moderately stable
Distillate	0.828	O–W	Unstable
Mineral seal	0.849	.....	Separates immediately
Distillate	0.857	W–O	Moderately stable
Paraffin oil	0.884	W–O	Stable
Cylinder oil	0.918	W–O	Stable

* O–W denotes the oil-in-water type; W–O, the water-in-oil type.

tant, as is illustrated by Table XXVII.[3] There is no explanation as to why the hydrocarbons behave in the manner shown in Table XXVII.

It has frequently been observed that neutral fat, for example lanolin (wool fat), will take up or bind considerable water. This property is probably due to its high sterol content, which favors a water-in-oil emulsion. The following results given by Degkwitz[4] illustrate this principle very clearly:

2 cc. triolein takes up 2.05 cc. H_2O
2 cc. triolein and 1 per cent cholesterol takes up 4.90 cc. H_2O
2 cc. triolein and 1 per cent cholesterol ester takes up 3.50 cc. H_2O
2 cc. triolein and calcium oleate takes up 4.20 cc. H_2O

Evidently the presence of cholesterol aids considerably in the absorption of water by triolein. This behavior is responsible for the erroneous statement that cholesterol is hydrophilic.

Theories. Only two of the many theories of emulsification will be considered: the solubility or interfacial tension theory which was originated by W. D. Bancroft, and the oriented molecular wedge theory developed independently by J. H. Hildebrand and W. D. Harkins. The Bancroft theory has been extended by Roberts.[5] According to this theory, in addition to the oil and water, there is a third phase, the emulsifier; and we must really consider two interfacial tensions, the oil-emulsifier and the water-emulsifier tension. If the oil-emulsifier

[3] Wm. Seifriz, Protoplasm, McGraw-Hill Book Co., New York, 1936.
[4] R. Degkwitz, Lipoide und Ionen, Theodor Steinkopff, Dresden, 1933.
[5] C. H. M. Roberts, J. Phys. Chem. **36**, 3087 (1932).

has a low interfacial tension, a water-in-oil emulsion results because it takes less work to form such an emulsion. Roberts claims to have measured these two interfacial tensions with a ring method and he finds the theory to hold. The interfacial tension theory is also in accord with the rule given above concerning the lyophilic or lyophobic nature of the emulsifying agent. It has been found that if the interfacial tension falls below 10 dynes per cm., an emulsion can be formed, and if it falls below 1 dyne per cm., the system emulsifies almost spontaneously.

The molecular wedge hypothesis of Harkins and Hildebrand had its origin in the work of Langmuir and Adam, on surface films of fatty acids. We have seen in an earlier chapter how fatty acids and soaps are of a polar nature. The carboxyl group is attracted by the water, and the paraffin chain by the oil; soap is thus orientated at an oil-water interface. If the soaps are the same size on both ends, they will, when packed closely together, assemble in a level plane; but if the ends are not the same size, then the plane will be bent, and the way it is curved will depend upon which end of the soap molecule is the larger. If, as with sodium soaps, the head which sticks in the water is the larger, the water surface will accordingly have the greater area and an oil-in-water emulsion will result. On the other hand, if we have a calcium soap, the paraffin end is the larger because a wedge is formed by the two paraffin chains with the calcium atom as the apex; the oil surface must therefore have the greater area, and a water-in-oil type will form. This theory at best is by no means a general one, as it could apply only to polar molecules whose ends are of unequal size. Then, too, a membrane formed by an emulsifying agent is usually much thicker than one molecule as is postulated by the theory. Another point to be considered is that the radius of curvature of an emulsion droplet would be practically zero when compared with molecular dimensions. The behavior of emulsions is extraordinarily unpredictable. Only living matter itself shows more idiosyncrasies than emulsions. This peculiarity is no doubt due to the very unstable nature of emulsions.

Phase Reversal. It is often possible to bring about phase reversal of emulsions stabilized by soaps. Soaps of the alkali metals favor an oil-in-water emulsion. On the other hand, the soaps of polyvalent metals favor a water-in-oil type. A reversal is noticed if we change the ratio of the "monovalent" soaps (Na) to the "polyvalent" soaps (Ca).

Table XXVIII illustrates the behavior of mixtures of olive oil and water in the presence of varying amounts of sodium hydroxide and calcium chloride.[6] The results shown in the table are no doubt due to the formation of calcium and sodium soaps.

[6] G. H. A. Clowes, J. Phys. Chem. **20**, 407 (1916).

TABLE XXVIII

PHASE REVERSALS

Cubic Centimeters of 0.1 *M* NaOH	Cubic Centimeters of 0.1 *M* $CaCl_2$			
	0.25	0.5	0.75	1.0
1.0	*R* *	W–O	W–O	W–O
2.0	O–W	R	W–O	W–O
3.0	O–W	O–W	R	W–O
4.0	O–W	O–W	O–W	R

* R denotes reversal; W–O, the water-in-oil type; and O–W, the oil-in-water type.

During reversal of emulsion, often one type persists while the other is being formed, so that compound emulsions result. An oil droplet which itself is part of an oil-in-water emulsion may contain a water-in-oil emulsion. Thus Seifriz[7] was able to prepare five emulsions in one, and, as he put it, like a chest of Chinese boxes.

Fernbacher[8] determined the critical ratios for the inversion point of an emulsion for a number of substances.

Lecithin : Cholesterol : : 1.1 : 1.0
Albumen : Cholesterol : : 0.9 : 1.0
Lecithin : Cholesterol ester : : 0.55 : 1.0
Albumen : Cholesterol ester : : 0.15 : 1.0
Na Oleate : Cholesterol ester : : 0.25 : 1.0.

Corran and Lewis[9] obtained much the same results.

The ratio of phospholipid to cholesterol is low in some tissues and indicates the probability of poor emulsification for these tissues. Some of these ratios are shown in Table XXIX. Bloor has often commented

TABLE XXIX

PHOSPHOLIPID: CHOLESTEROL RATIO FOR SEVERAL TISSUES

Organ	Phospholipid : Cholesterol Ratio
Brain	2.5
Liver	18.0
Heart	16.0
Kidney	11.0
Serum	2.5
Muscle	26.0
Lung	60.0

[7] Wm. Seifriz, Am. J. Physiol. 66, 124 (1923).
[8] Fernbacher, dissertation, 1932, cited by Degkwitz, "Lipoid und Ionen."
[9] J. W. Corran and W. C. Lewis, Biochem. J. 18, 1364 (1924).

on the parallelism between the "activity of a tissue" and its phospholipid: cholesterol ratio. Bloor and Snider[10] were able to show that, the higher the activity of a muscle, the greater was the phospholipid: cholesterol ratio. These authors believe this to be evidence that phospholipid functions as an aid in metabolism and suggest that it acts as an oxygen carrier. It has been found by other workers that the physiological activity of the corpus luteum is associated with increasing amounts of phospholipid and usually, also, free cholesterol; degeneration or retrogression is characterized by falling values for phospholipid and increasing amounts of cholesterol and neutral fat.

It is possible to offer an explanation for the dependence of lipid metabolism on the phospholipid: cholesterol ratio on a purely physicochemical basis. Whether or not it is the correct one is another matter; it is better to have some kind of theory, however, than none at all. It is clear that a high phospholipid: cholesterol ratio is connected with a catabolism of fat, and a low ratio is associated with fat accumulation. The feeding of cholesterol to an animal, for example, leads to the accumulation of fats in the liver, and it is reported that this condition can be prevented by the feeding of choline and lecithin.[11] It is possible that the accumulation of lipids in the presence of cholesterol is due to the poorer emulsification of the fat, resulting both in a greater conversion of carbohydrate to fat, and also in poorer transportation of the fat, owing to the increased size of the emulsion particles. The feeding of choline would stimulate lecithin formation, which would improve the emulsion and increase the surface of the fat, which would lead to an increase in its utilization as well as to an increase in transportation—all resulting from a decrease in size of the fat droplets. This would explain why the activity of a tissue varies directly with its phospholipid: cholesterol ratio, and why phospholipid is associated with the metabolism of the fats. This, no doubt, is not the only rôle which phospholipid can and does play. For example, it may act as an oxygen carrier. But, according to this theory, we see it acting in a dual rôle, as part of the structure of protoplasm, *i.e.*, as an emulsifier in the tissue cells, and through this rôle as an enhancer of lipid metabolism. It will be recalled that pure lecithin is a fairly poor emulsifier, but when combined with protein, it is extremely capillary-active. In the cell, it no doubt occurs largely combined with protein.

On first thought, it might seem that the administration of any good emulsifying agent which favored an oil-in-water emulsion and was not toxic to an animal would increase the fat dispersion and bring about

[10] W. R. Bloor and R. H. Snider, J.B.C. **107**, 458 (1934).
[11] C. H. Best and H. J. Channon, Biochem. J. **29**, 2651 (1936).

these results, but this is not true because the emulsifying agent would have to penetrate the wall of the cell containing the fat, which gum acacia, for example, could not do. It is not suggested that there is an actual inversion of the fat emulsion, except perhaps in extreme cases, but simply a coarser emulsion in the case of high cholesterol with larger, inert globules of fat. This theory is beautifully supported by the recent work of MacLachlan,[12] who found with germinating soy bean seeds that the percentage of free cholesterol in the cotyledons decreased very sharply, which is, no doubt, connected with the fat metabolism in the cotyledons, as is shown by the drop of the total fatty acids from 16.8 per cent in the ungerminated seeds to 6.49 to 10.32 per cent in the cotyledons. It would be most interesting to follow the change in the phospholipid under these conditions.

A study of the phospholipid and cholesterol during seed formation would also be very important. What is needed above all, however, is a separation of the cholesterol into free cholesterol and cholesterol ester on one hand, and the phospholipid into at least cephalin and lecithin on the other. The free cholesterol is certainly very much different from cholesterol ester in its physiological as well as its emulsifying power. Lecithin and cephalin probably play markedly individual rôles. It is even stated by Degkwitz that cephalin is a water-in-oil emulsifier.

LIPIDS AND CELL PERMEABILITY

Various workers have suggested at one time or another that the lipids are involved in cell permeability. Overton,[13] in a long series of articles, proposed a general rule and developed a theory regarding the permeability of cells. He says, "Die Grenzschichten des Protoplasmas der Zellen von einer fettartigen Substanz, und zwar speciall von einem. Gemisch von Lecithin cholesterin, imprägniert sind und dass das schnellere oder langsamere Eindringen der einzelner Verbindungen in die Zellen—von ihrer Löslichkeit in diesem Lecithin-cholesterin Gemisch oder vielmehr von ihrem Teilungskoeffizienten zwischen Wasser und diesem Gemisch abhängig ist." Overton attempted to test his theory by comparing the partition coefficient of various compounds between water and lipids with the ease of permeability of these compounds through the cell walls. Numerous workers have investigated the problem since Overton's pioneer studies, without, however, obtaining any really conclusive evidence. The theory seems to hold for some cells. For example, Jurisic[14] found that results with red blood cells were in

[12] P. L. MacLachlan, J.B.C. **113**, 197 (1936); **114**, 185 (1936).

[13] E. Overton, Z. physik. Chem. **22**, 186 (1896); Jb. Bot. **34**, 669 (1900); Pflügers Arch. **92**, 115 (1902), **92**, 346 (1902), **105**, 176 (1904).

[14] P. J. Jurisic, Biochem. Z. **181**, 17 (1927); **196**, 223 (1928).

good agreement with the theory. On the other hand, certain cells such as young cells from the intestines and eyes were quite permeable to lipid insoluble dyes. There are other difficulties. All cells are permeable to water, and animal membranes do not obey Overton's rule.

Nirenstein[15] developed an experimental model which gives results more nearly like those obtained *in vivo*. He used a mixture of oil, oleic acid, and a base (diamylamin).

It must be added that our ideas concerning the phospholipids have changed since Overton proposed his theory. We know now, for example, that they are capable of becoming hydrated and perhaps of transporting water in this fashion. Cell membranes certainly contain both lipids and proteins. It has been suggested that the cell membrane has a mosiac structure, part being protein and part lipid, thus allowing permeability to both lipid-soluble and lipid-insoluble material.

Mudd[16] found by means of electroendosmosis that the isolectric point of mammalian serous membranes corresponds closely to what one would expect the isoelectric point of proteins to be. The electroendosmosis probably took place between the cells, however, and not through the cells. Then, too, the isoelectric point of lecithin is still a doubtful quantity from which to argue. Taylor *et al.*,[17] in a series of studies on tastes, found their results in accord with the idea that the taste buds are essentially fatlike in nature rather than proteinlike.

Clowes[18] elaborated on Overton's theory. He believed that the cell membrane is made up of a mixture of oil-in-water and water-in-oil emulsions and the permeability of the cell is dependent upon the balance of these emulsion systems. Thus an oil-in-water emulsion would be permeable to water-soluble substances, and conversely. He was able to demonstrate phase reversal *in vitro* by the addition of calcium chloride to an oil-in-water emulsion stabilized with a sodium soap. It has been shown, however, that phase reversal is difficult if proteins and lecithin are present. Clowes believed that this phase reversal might be the cause of ion antagonism between calcium and sodium, so generally observed in biology. Investigations along these lines have been singularly unproductive, which leads one to suspect that the ideas are either untrue or only partly true. Cell membranes have considerable rigidity and toughness—properties which emulsions and mixtures of lipids do not possess. Ascherson,[19] long ago, suggested that cell membranes are made at least

[15] E. Nirenstein, Pflügers Arch. **179**, 233 (1920).
[16] S. Mudd, J. Gen. Physiol. **9**, 73 (1925).
[17] N. W. Taylor, *et al.*, J. Gen. Physiol. **11**, 207 (1928); Protoplasma **10**, 98 (1930), **10**, 84 (1930), **4**, 1 (1928).
[18] G. H. A. Clowes, J. Phys. Chem. **20**, 407–451 (1916).
[19] F. M. Ascherson, Arch. Anat. Physiol. **1840**, 44.

partly of surface-denatured protein, at any rate that such denatured protein is the structural element in cell membranes. Such a membrane would form spontaneously and would exhibit all the physical properties of cell membranes as we know them. It would seem that permeability studies would be greatly furthered by a detailed chemical study of cell membranes.

NATURAL EMULSIONS

There are at least three important and well-recognized natural emulsions. These are milk, latex from certain plants, and chylomicron emulsion.

Milk. Milk is an excellent example of an oil-in-water emulsion. It presents many points of extreme physicochemical interest. It is essentially a colloidal system containing inorganic salts, sugar, and protein, with the fat in coarse dispersion or suspension. It is beyond the scope of this book to discuss the physiology of milk secretion.

The composition of milk fat is peculiar, as it contains in appreciable amounts a wide range of fatty acids, all the way from the C_4 butyric to the C_{20} arachidic. It appears as if the mechanism for fat synthesis had partly failed, and released a number of unfinished products.

The fat globules in milk range from 1 micron to 22 microns in diameter. The major part are, however, 2 to 3 microns in diameter. Prieger[20] reports the isoelectric points of these milk globules to be between a *p*H 3.95 and 4.2. The question naturally arises as to the covering or emulsifying agent involved.

Palmer *et al.*[21] have made some interesting studies on the membrane material surrounding the milk fat globules. The membrane material was obtained by washing cream with distilled water, churning this cream, and, under diminished pressure, evaporating the buttermilk which contained the surface-active membrane compounds. The residue was exhaustively extracted with alcohol and ether to remove the lipids. The protein was found not to correspond to any of the other milk proteins and was regarded as a new protein. The total nitrogen was very low (*ca.* 12 per cent). The lipid fraction consisted of high-melting glycerides and also of lecithin, cephalin, and a certain amount of sphingomyelin. The original protein-lipid mixture had an isoelectric point of *p*H 3.9 to 4.0, which confirmed its identity as the membrane material on the milk droplets.

Palmer and Tarassuk[22] found that this membrane material is

[20] I. Prieger, Biochem. Z. **217**, 331 (1930).

[21] L. S. Palmer and H. F. Wiese, J. Dairy Sci. **16**, 41 (1933); C. E. Rimpila and L. S. Palmer, J. Dairy Sci. **18**, 827 (1935); H. F. Wiese and L. S. Palmer, J. Dairy Sci. **17**, 29 (1934).

[22] L. S. Palmer and N. P. Tarassuk, J. Dairy Sci. **19**, 323 (1936).

responsible for the low curd tension of fresh, sweet-cream buttermilk, and they apparently feel that the protein fraction of the membrane material is more important than the lipid fraction in producing this curd tension. Whether this is also true in the emulsification of the milk droplets is not known.

Latex. A large variety of plants (*Fiscus elastica, Hevea braziliensis, Euphorbia, Solidago,* etc.) produce an emulsion known as latex, which consists of dispersed globules of hydrocarbon, stabilized by protein and resinous matter. Crude rubber can be obtained if the emulsion is coagulated. The hydrocarbon to which rubber owes its elastic properties is probably a derivative of isoprene.

Recently Moyer[23] has studied the nature of the membrane material of latex from a large number of *Euphorbia.* Using electrophoretic measurements and the Mudd interfacial technique, he came to the conclusion that in some specimens the membrane material was almost entirely protein and in others made up of a material which he later identified as being of a sterol nature. Incidentally he was able to use the electrophoretic measurements as a means of classifying the *Euphorbia,* indicating a very specific surface for the latex of each variety of plant.

Chylomicrons. The fat is transported in the blood as fine particles called the chylomicron emulsion by Gage and Fish,[24] who conducted an extremely interesting series of studies on the absorption and transportation of fat in blood. They used the dark-field microscope to make the chylomicrons visible and also utilized fat-soluble dyes to follow the fat through the body. The fat-soluble dye was found to associate itself with the fat fed and to be carried along by the fatty acid. It was even deposited in hens' eggs, thus showing that the fatty acids fed were utilized by the hen in the building of the egg. The chylomicrons range from 1 to 0.5 micron in diameter. They increase slightly in size during digestion and absorption. Only 60 per cent of the fat ingested appears in chylomicron form. Dye was not found in the milk fat of cows after being fed with food containing butter fat. This indicates that the milk fat of cows does not come directly from the fat ingested. Carnivorous and omnivorous animals, on the other hand, did carry the dye through into the milk. Almost immediately on feeding, the fat began to be absorbed but it was not until about nine hours later that the process was complete. The time involved was largely independent of the amount of fat fed. Fig. 62 shows the variation of the chylomicrons in blood after feeding.

[23] L. S. Moyer, Botan. Gaz. **95**, 678 (1934); Am. J. Botany **21**, 293 (1934), **22**, 609 (1935).

[24] S. H. Gage and P. A. Fish, Am. J. Anat. **34**, 1 (1924).

It is interesting to consider the stabilizing agent for this emulsion. Is it of a phospholipid or of a protein nature? Ludlum, Taft, and Nugent[25] endeavored to answer this question by determining the isoelectric point of the chylomicron emulsion and comparing it with the isoelectric point of the phospholipids and proteins. They found an isoelectric point for the chylomicrons at a *p*H of 5. They concluded from this that the chylomicrons were covered with a mixture of globulin and albumin. Their conclusions must be taken with reservation, however, since the isoelectric point of naturally occurring lecithin is at least 3.9 and may be as high as 6.9. The question must be regarded as still undecided. Bloor found the phospholipid content of the blood to increase during fat absorption. This may indicate that the phospholipids play a rôle in the emulsification of the neutral fat, or this increase may simply be incidental to the process. The chylomicron count should serve as a valuable agent in estimating the amount of fat in the blood. That a paralellism exists is shown in Fig. 63.

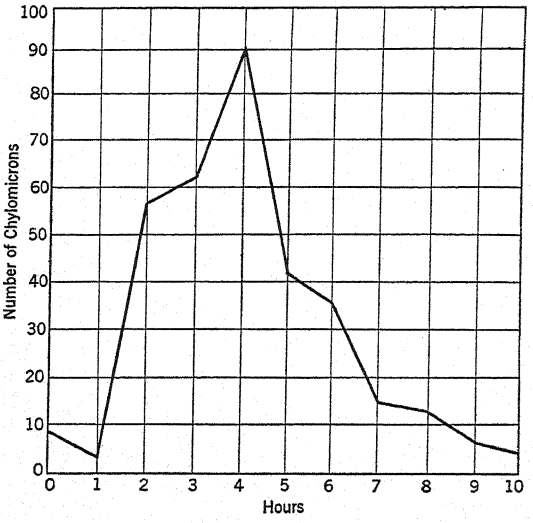


FIG. 62.—Variation of the chylomicron count in blood after feeding.

Sullman and Verzar[26] were able to show that about 25 per cent of the plasma lipids were diffusible through collodion membranes. It can be seen from Fig. 63, from Ludlum, Taft and Nugent, that if the straight line is extrapolated to zero content of chylomicrons, 100 cc. of plasma still hold 50 mg. of ether-soluble material. These 50 mg. probably correspond to the diffusible lipids found by Sullman and Verzar. MacArthur[27] reports a correlation coefficient of 0.46 ± 0.24 between the total lipids of dog plasma and the chylomicron count.

[25] S. D. Ludlum, A. E. Taft, and R. L. Nugent, Colloid Symposium Annual **VII**, 233 (1930).

[26] H. Sullman and F. Verzar, Biochem. Z. **270**, 44 (1934).

[27] E. H. MacArthur, Proc. Soc. Exptl. Biol. Med. **28**, 555 (1931).

Bloor[28] found that the blood plasma in the post-absorptive period is normally clear although containing appreciable amounts of cholesterol, cholesterol esters, lecithin, and, probably, also, some fat. The introduction of more fat into the blood, whether by feeding or by mobilization of the fat stores, generally produces a milkiness normally lasting only a few hours, but in certain conditions, mainly pathological, it may persist for longer periods. The milkiness of the plasma is termed lipemia. In humans the plasma contains 0.5 to 0.8 gram of lipid per 100 cc., and lipemia ordinarily appears when values above these figures, but generally below 1 gram per 100 cc., are reached, although figures as high as 4.35 grams have been reported with the plasma still clear.

By far the greatest incidence of persistent lipemia has been found in diabetics, although sufferers from nephritis and chronic alcoholism furnish some notable examples. Diabetics sometimes have amazingly high blood fats, reaching in some individuals as high a value as 25 per cent, which is, of course, a thick cream. It was discovered by Boggs and Morris that a high-grade lipemia can be produced in rabbits by bleeding. A bleeding lipemia can be produced on a fat-free diet. The marrow of the long bone must be emphasized as a possible source of the fat. In general: (*a*) in lipemia there is always an increase of lecithin and cholesterol as well; (*b*) at the height of the lipemia, the greatest increase is in the fat, the next in the cholesterol, and least in the lecithin. As a result, the lecithin: cholesterol ratio is generally below normal. Persistent lipemia may be produced by fat from either fat depots or the food, and Bloor believes that lipemia may arise either as the result of the inability to metabolize or because of a large influx of fat.

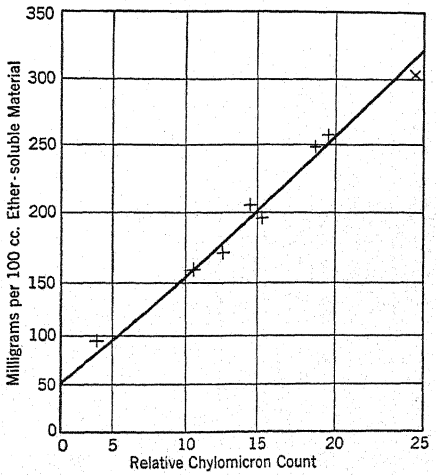


FIG. 63.—Ether-soluble material of blood plasma as a function of the relative chylomicron count.

Fat Embolism. In fat embolism, part of the fat is eliminated in the urine. Large emboli undoubtedly lead to rupture of the capillary walls, and probably some fat also escapes through stomata or similar inter-

[28] W. R. Bloor, J.B.C. 49, 201 (1921).

cellular openings. Some may escape in the bile, and some is probably taken up by the tissue and endothelial cells by phagocytosis. No doubt it is acted upon by the lipase of the plasma. Fat emboli first lodge in the lung tissue. As much as 2 to 6.5 per cent of the fat of venous blood has been found to be emboli. In many instances the emboli come from fractured bones; occasionally, as a result of surgical operations. Frequently they are fatal. There is evidence to believe that fat embolism is due to loss of the emulsifying power of the blood, resulting in the breaking of the chylomicron emulsion with disastrous results. It has been reported that, if gum acacia is injected, then it is possible to inject large quantities of oil without embolism. Hoffheinz[29] has recently written a book dealing with fat and air emboli.

Davis and Goodchild[30] have commented on the problem of fat embolism in relation to the breaking of the normal fat emulsion in the blood.

Through an error, the important and interesting work of Sinclair relative to the rôle of phospholipids in fat absorption through the intestinal mucosa was omitted from page 129 under the discussion of the physiology of phospholipids. The author wishes to apologize for this oversight.

Sinclair[31] fed cats oils with low and high iodine number. He then determined the weight and iodine number of the phospholipid fatty acids of the intestinal mucosa and muscles of the intestines. He found the weight of the phospholipids to be constant. The iodine number of the phospholipid fatty acids, however, followed that of the ingested fat. Those of the intestinal muscle did not. Sinclair interpreted these results to mean that the phospholipids play a rôle in the resynthesis of the fat, and that the absorbed fatty acids must pass through the phospholipid stage before it is built into neutral fat. That is

$$\text{Fatty acid} \rightarrow \text{phospholipid} \rightarrow \text{neutral fat}$$

In support of Sinclair's theory are the results of Vezar and Laszt,[32] who found that the feeding of glycerol and phosphate and, to a far greater extent, glycerophosphoric acid increased the absorption of oleic acid.

It has been known for many years that after the fat is synthesized in the mucosa, it enters the blood stream to the extent of 60 per cent by way of the larger lymphatics of the mesentary, thence to the recep-

[29] S. Hoffheinz, Die Luft und Fettembolie, Ferdinand Enke Verlas, Stuttgart (1933).

[30] H. L. Davis and C. G. Goodchild, J. Chem. Education **13**, 478 (1936).

[31] R. G. Sinclair, J.B.C. **82**, 117 (1929).

[32] Vezar and Laszt, Biochem. Zeit. **270**, 24 (1934).

taclum chyli and then by way of the left thoracic duct, and enters the blood at the junction of the left subclavian and jugular veins.

The other 40 per cent presumably enters the blood capillaries directly and is conveyed by the blood to the various organs. The difference between the two routes means that fat passed directly into the blood will go through the liver before it reaches other tissues, and that which enters through the thoracic duct will be distributed equally to all parts.

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